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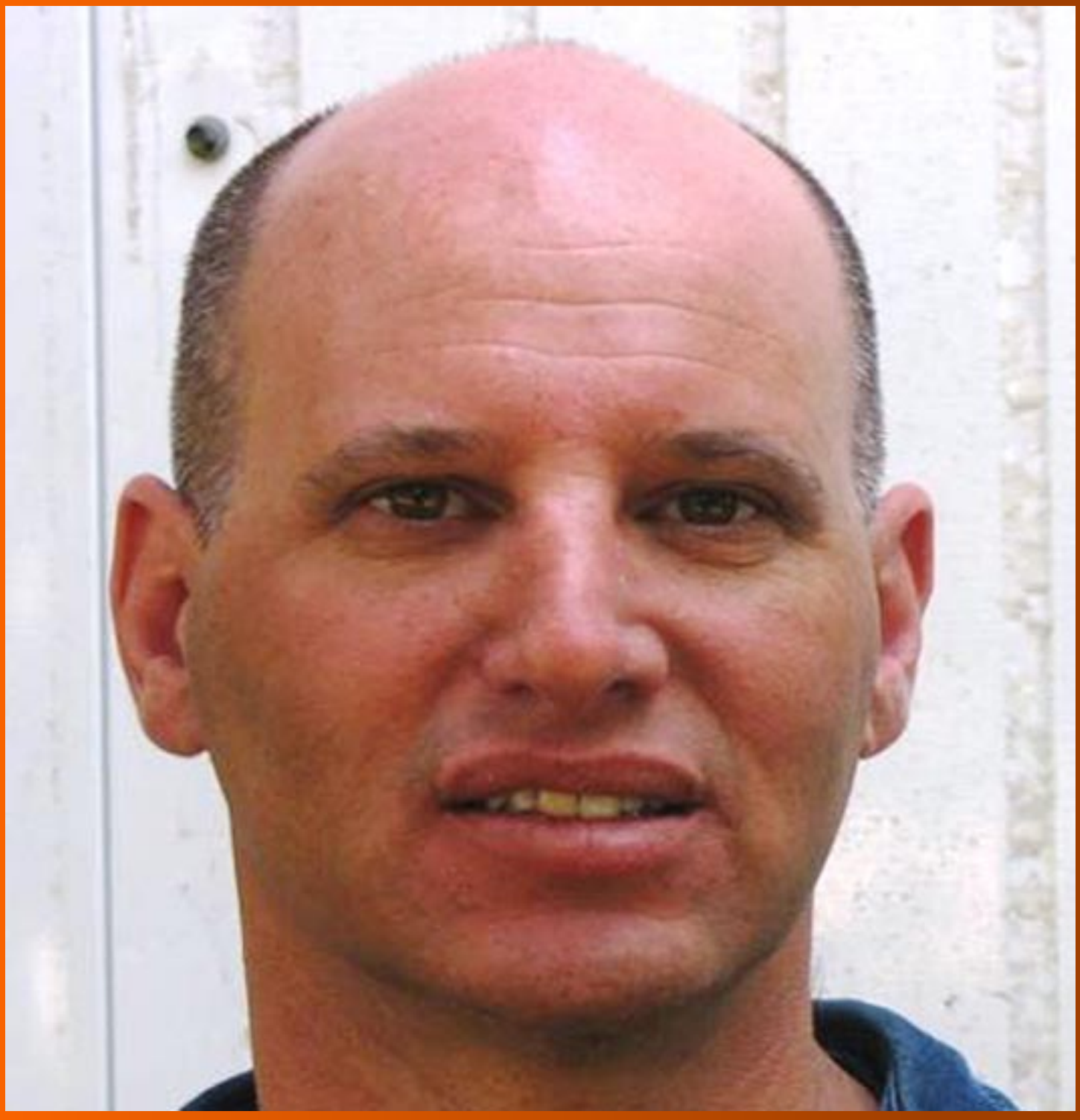

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Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Clinical Infectious Diseases
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
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REVIEW

Treatment of methicillin-resistant *Staphylococcus aureus* infections: Importance of high vancomycin minumum inhibitory concentrations

Alejandra Morales-Cartagena, Antonio Lalueza, Francisco López-Medrano, Rafael San Juan, José María Aguado

Alejandra Morales-Cartagena, Antonio Lalueza, Francisco López-Medrano, Rafael San juan, José María Aguado, Infectious Diseases Unit, Department of Medicine, University Hospital 12 de Octubre, 28041 Madrid, Spain

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Correspondence to: Dr. Alejandra Morales-Cartagena, Infectious Diseases Unit, Department of Medicine, University Hospital 12 de Octubre, Av. Córdoba km 5.400, 28041 Madrid, Spain. a.morales.cartagena@gmail.com
Telephone: +34-91-3908247
Fax: +34-91-3908112

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Abstract

Staphylococcus aureus (SA) infections remain a major cause of morbidity and mortality despite the availability of numerous effective anti-staphylococcal antibiotics. This organism is responsible for both nosocomial and community-acquired infections ranging from relatively minor skin and soft tissue infections to life-threatening systemic infections. The increasing incidence of methicillin-resistant strains has granted an increasing use of vancomycin causing a covert progressive increase of its minimum inhibitory concentration (MIC) (dubbed the MIC "creep"). In this way, the emergence of vancomycin-intermediate SA (VISA) strains and heteroresistant-VISA has raised concern for the scarcity of alternative treatment options. Equally alarming, though fortunately less frequent, is the emergence of vancomycin-resistant SA. These strains show different mechanisms of resistance but have similar problems in terms of therapeutic approach. Ultimately, various debate issues have arisen regarding the emergence of SA strains with a minimum inhibitory concentration sitting on the superior limit of the sensitivity range (*i.e.*, MIC = 2 μg/mL). These strains have shown certain resilience to vancomycin and a different clinical behaviour regardless of vancomycin use, both in methicillin-resistant SA and in methicillin-sensitive SA. The aim of this text is to revise the clinical impact and consequences of the emergence of reduced vancomycin susceptibility SA strains, and the different optimal treatment options known.

Key words: *Staphylococcus aureus*; Minimum inhibitory concentration; Methicillin-resistant *Staphylococcus aureus*; Vancomycin-intermediate *Staphylococcus aureus*; Heteroresistant-vancomycin-intermediate *Staphylococcus aureus*; Vancomycin resistant *Staphylococcus aureus*

Core tip: The emergence of increasing vancomycin-resistance in *Staphylococcus aureus* (SA) isolates, has stirred up the basis of therapeutic approach in staphylococcal infections. Complete vancomycin-resistance is acquired through plasmid transmission of enterococcal gene *vanA*. However, the development of strains with gradual loss of vancomycin-susceptibility

seems to be related to conformational bacterial changes and affects its pathogenicity and even its susceptibility to other antimicrobials (other than vancomycin). It has been observed that the impact of diminished vancomycin susceptibility could not only affect methicillin-resistant SA but has also been related to worse prognosis in methicillin-sensitive SA infections. There is yet much to explore to better define the impact of higher vancomycin minimum inhibitory concentration in staphylococcal infections.

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STAPHYLOCOCCUS AUREUS, AN EVOLVING AGENT

Little after the beginning of the antibiotic era came the arrival of antibiotic resistance. The first *Staphylococcus aureus* (SA) strains resistant to penicillin appeared in 1942 due to an inducible beta-lactamase, and since then it has been evolving, developing resistance to most other antibiotics used for staphylococcal infections[1]. In 1959 methicillin became the best option to surpass penicillin resistance, however, resistance appeared only 2 years later [methicillin-resistant *Staphylococcus aureus (*MRSA)][2]. It took some years to spread, and not until the mid 1980's did MRSA reach alarming figures[3-5].

The mechanisms leading to methicillin resistance involve the expression a chromosomal gene *mecA*, which is found in the staphylococcal cassette chromosome (SCC), a mobile genomic element. This gene encodes penicillin binding protein 2a (PBP2a) that has a low affinity for certain betalactams, including penicillin and methicillin. The origin of methicillin resistance is uncertain, however, studies up to now suggest that it first appeared in coagulase-negative staphylococci, and then was transferred to methicillin-susceptible *Staphylococcus aureus* (MSSA) through horizontal gene transfer. Genes encoded in SCC*mec* have proved to be decisive in antibiotic resistance, however, it is not clear whether the play any relevant role in *S. aureus* virulence[6].

Whereas methillin-resistance developed in the years following its discovery, *S. aureus* strains showing reduced susceptibility to vancomycin were not described until 1997. However, many reports of similar findings started to appear shortly after[7]. Even if vancomycin resistance expansion has taken a different form than other patterns of antibiotic resistance and perchance less aggressive, it is a growing problematic in staphylococcal infections and deciding the optimal treatment approach is an on-going challenge.

EPIDEMIOLOGY OF AS INFECTION

Incidence of methicillin resistant SA

MRSA has spread like an epidemic, becoming 41.2% of the strains isolated in Europe at the present time[8]. More than 25% of *S. aureus* strains isolated in Spanish hospitals are methicillin resistant[9]. Prevalence of MRSA in Asian hospitals are globally very high, reaching 60% of the SA isolates in countries like Southern Korea, Vietnam or Taiwan[10,11]. In the United States, studies in the last decade declared that more than 94000 MRSA-associated infections occur every year, with an estimation of 18650 MRSA-infections attributable deaths[12]. One of the most important risk factors in developing MRSA infection has been observed to be MRSA colonization, detected through positive nasal-carriage. In a recent meta-analysis evaluating the prevalence of MRSA colonization and infection in patients admitted to the intensive care unit (ICU) (studies included from Europe, North and South America, Asia and Australia) they observed a prevalence of MRSA colonization ranging from 5.8% to 8.3%, which was higher in North American studies, with and upward trend. MRSA colonization was found to be associated with an important increased risk for MRSA infections [relative risk (RR) of 8.33][13].

Lately however, decreasing trends in hospital-onset MRSA infections have been observed in several surveillance studies. In an observational study of all Department of Defence TRICARE beneficiaries from January 2005 to December 2010, they found that annual rates of both community-onset and hospital-onset MRSA bacteraemia decreased (from 0.7 per 100000 person-years in 2005 to 0.4 per 100000 person-years in 2010)[14]. In addition, MRSA central line-associated bloodstream infections have been decreasing in United States intensive care units[15]. Decline in healthcare-associated invasive MRSA infections have also been recently reported[16]. The emergence of community-associated MRSA (CA-MRSA) and its introduction into healthcare settings has changed the epidemiology of *S. aureus* infection in the American continent and worldwide. These isolates are chiefly associated with a wide range of soft tissue infections and are sometimes implicated in severe pneumonias. They are rarely encountered in patients with bacteraemia. In an observational study to analyse the impact of CA-MRSA emergence on *S. aureus* bacteraemia (SAB), they describe a steadily decreasing rate of SAB both for community-associated (especially MSSA bacteraemia) and hospital-onset cases, whereas the rate of community-onset healthcare-associated cases did not change[17]. These results emerge in the context of multiple strategies adopted with the objective of reducing device-related and surgical-site infections in hospital settings. Most of these studies and revisions are based on retrospective data and observational evidence, and must therefore be weighed in this

context.

The vast majority of published epidemiological studies about the prevalence and clinical impact of SA infections refer to the American and European continents. There is scarce information about *S. aureus* epidemiology in non-Western parts of the world (Africa, Middle East, Asia and Oceania) as highlighted in Rasigade's review[18].

Morbidity and mortality associated to MRSA infections

MRSA bacteraemia is associated with a considerable mortality. In a recent study that took place in nine different areas of the United States where they analysed almost 9000 MRSA invasive infections, bacteraemia (75%) was the clinical syndrome most frequently associated with invasive MRSA infection. Standardized mortality rate in this study was 6.3 per 100000 (interval estimate 3.3-7.5)[12].

Given that MRSA infections have been historically mainly healthcare-associated, bacteraemia by these pathogens have been found more frequently in patients who are severely ill or with a great number of comorbidities. Thereby there has been a continuing perception that this organism is particularly virulent. However, its virulence compared to that of MSSA remains controversial[19]. Earlier studies and meta-analysis described an almost two-fold increase in mortality in patients with MRSA bloodstream infections than those due to MSSA[19]. However, other studies analysing healthcare-associated MRSA bacteraemia in that same period and in the following years found no differences[20-22]. In a meta-analysis that evaluated the results of 9 international studies comparing MRSA *vs* MSSA risk factors and mortality, they observed that the risk of death was higher in patients with MRSA bacteraemia than those with MSSA bacteraemia in all but one of the studies, with a RR of death of 2, ranging from 0.89 to 4.94. They described potential risk factors associated to MRSA bacteraemia, such as: prior antibiotic therapy, longer previous hospital stay, older age, male sex, past history of MRSA infection and admittance or treatment in an ICU[23]. However once again, this almost two-fold higher mortality risk, seemed to be interfered by the base-line comorbid and nosocomial situation of those patients[19,23].

ROLE OF VANCOMYCIN IN METHICILLIN-RESISTANT *S. AUREUS* INFECTIONS AND CONSEQUENCES OF ITS USE

The use of vancomycin, a glycopeptide discovered in 1952 and approved shortly after, didn't spread until years later with the emergence of pseudomembranous enterocolitis and the spread of MRSA infections[24]. Its mechanism of action consists on the inhibition of the bacterial wall synthesis, with a slow bactericidal effect compared to beta-lactams[24]. Nephrotoxicity is its main toxicity concern, needing caution for patients with renal impairment. In these cases, if treatment with vancomycin is unavoidable, the best possible approach would be to confirm serologic levels stay within optimal concentration for bactericidal activity (see section "Risk of nephrotoxicity with elevated vancomycin doses")[25].

Clinical guidelines still recommend intravenous vancomycin as one of the first choice antibiotic therapies for the treatment of MRSA infections including bacteraemia, infective endocarditis, meningitis, central-line associated infection, septic thrombosis, osteomyelitis, and septic arthritis (in the latter, the addition of rifampin is sometimes considered). In specific severe complications such as severe septic shock, toxic syndrome, or necrotizing pneumonia, some experts consider adding adjunctive therapy with clindamycin or linezolid, which are protein synthesis inhibitors. Intravenous immunoglobulin have also shown good results in these situations[26].

Unlike with other antibiotics, *S. aureus* did not start to show resistance to vancomycin until 40 years after its discovery. In 1996 in Japan, the first vancomycin-intermediate *Staphylococcus aureus* (VISA) isolate was reported[27,28], and subsequently heteroresistant VISA (hVISA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) isolates were described (see section "Minimum inhibitory concentration value for vancomycin and mechanisms of resistance"). Thereafter, vancomycin failure and apparently worse clinical outcome in these staphylococcal infections started to be described[29]. In the Asian continent VISA has never disseminated widely and has only been sporadically reported. VRSA, on the other hand, with greater MIC than 16 μg/mL (harbouring gene *vanA*), have been increasingly reported from northern India and West Bengal, both in clinical and colonization isolates[11].

Minimum inhibitory concentration value for vancomycin and mechanisms of resistance

More than 20 years ago, the Clinical and Laboratory Standards Institute (CLSI) first set broad minimum inhibitory concentration (MIC) cut-off point and disk diffusion testing of vancomycin in *S. aureus* isolates (resistance set at $\geqslant$ 34 μg/mL). In 1998, after the appearance of the first *S. aureus* strains with reduced vancomycin susceptibility, they lowered the disk diffusion breakpoints, in order to detect these strains to $\leqslant$ 4 μg/mL. However, clinical failures with vancomycin in patients with MRSA infections resulted in a re-evaluation of its MIC breakpoints in 2004. Finally in 2006 the CLSI established vancomycin MIC susceptibility cut-off point in $\leqslant$ 2 μg/mL, 4-8 μg/mL for VISA and finally $\geqslant$ 16 μg/mL for VRSA. MIC for VRSA was lowered to 16 μg/mL because MICs above that limit had shown high probability of adverse clinical outcome[30]. Even with these changes, concerns about the declining susceptibility to glycopeptides in MRSA infections persisted[31].

S. aureus cell wall is composed of layers of murein monomers (peptidoglycan) with D-alanine-D-alanine

(D-ala-D-ala) residues. From the cytoplasm, where these monomers are synthesized, a lipidic transporter (lipid Ⅱ) transfers them through the membrane. It is then built into the peptidoglycan chain by enzymes situated within the membrane. Vancomycin binds to these D-ala-D-ala residues and blocks the assembly of peptidoglycan monomers, stopping bacterial growth[32]. VISA and VRSA have shown to have different mechanisms of resistance. In the case of VISA, it has been observed that they form a thickened cell wall, with added peptidoglycan layers, and therefore vancomycin isn't able to saturate its target nor reach to the surface of the cell wall, and becomes entrapped within it, never attaining its disruption[33,34]. The term glycopeptide-intermediate SA is sometime used in these strains, given that they frequently show similar patterns of resistance for teicoplanin. Though most VISA strains are also methicillin-resistant, a minority do show susceptibility to methicillin[6]. Intermediate vancomycin resistance has been associated to previous exposure to vancomycin and it seems these isolates can regain vancomycin susceptibility when the antibiotic pressure is withdrawn[35].

VRSA, with MIC breakpoint ⩾ 16 μg/mL considering current standards (CLSI) was first detected in 2002. Fortunately it is yet extremely uncommon[36]. VRSA acquire their mechanism of resistance from a gene transferred from vancomycin-resistant enterococci, gene *vanA* (usually transferred by transposon plasmids-Tn*1546*). The resistance mechanism relays on the change of a peptidoglycan residue (D-ala-D-ala by D-ala-D-lactate), so that vancomycin is not able to bind to exert its blockage of the wall synthesis. VISA strains do not carry *vanA*, *vanB*, or *vanC* genes[7,37].

Little after the description of VISA, arose the observation of subpopulations of MRSA apparently vancomycin-susceptible, showing atypical glicopeptide-resistance patterns, referred to as hVISA. These isolates would fall one step before VISA, in vancomycin resistance. Patients with hVISA were found to have been usually exposed to vancomycin in lower levels than desired therapeutic objectives (*i.e.*, < 10 μg/mL). The population analysis profile-area under the curve (AUC) calculation is the reference method to identify hVISA strains, which is an arduous process and is not always available in all laboratories. Measuring vancomycin-MIC values of these subpopulations, most of them will show a MIC ⩾ 2 μg/mL, but some yet show MICs < 2 μg/mL. This increases the difficulties involved in their correct identification. There is evidence of prevalence increase of hVISA strains in selected locations. It has been observed that patients with complicated MRSA infections might be at a greater risk of hVISA. In this context, in a recent international study they found that 29% of the patients with MRSA infective endocarditis had isolates with hVISA subpopulations[24,36]. Vancomycin heteroresistant *S. aureus* have been classified with a MIC ranging from 2 to 8 μg/mL.

Table 1 *Staphylococcus aureus* glycopeptide minimum inhibitory concentration cut-off values (μg/mL) as defined by Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing (determined by broth microdilution)

Antibiotic	CLSI (2011)			EUCAST (2011)	
	S	VISA	R	S	R
Vancomycin	⩽ 2	4-8	⩾ 16	⩽ 2	> 2
Teicoplanin	⩽ 8	-	⩾ 32	⩽ 2	> 2

CLSI: Clinical and Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; S: Sensitive; VISA: Vancomycin-intermediate *Staphylococcus aureus*; R: Resistant.

It has also been recently observed that there are emerging *S. aureus* strains with a MIC sitting on the superior limit of the sensitivity range (*i.e.*, MIC > 1.5 μg/mL), that could show certain resilience to vancomycin and a different clinical behaviour[31,38,39].

This resistance or loss of sensitivity to glycopeptides has given rise to plenty of debates regarding its clinical and epidemiologic relevance. As previously mentioned, partly due to the concerns of reduced vancomycin efficacy, in 2006 the CLSI lowered the *S. aureus* vancomycin-susceptible MIC cut-off point from 4 μg/mL to 2 μg/mL[30]. This cut-off point value is shared by the European Committee on Antimicrobial Susceptibility Testing, yet they do not hold the same MIC classification for VISA strains (which CLSI classify with MIC from 2-4 to 8 μg/mL) and VRSA (MIC ⩾ 16 μg/mL), and consider all *S. aureus* strains with MIC higher than 2 μg/mL, as "clinically" resistant to vancomycin (Table 1).

Different microbiologic methods to calculate vancomycin MIC

Detection of VISA strains can be difficult, and they may take more than two days of incubation to grow on culture plate[7]. Quantitative antimicrobial susceptibility methods are the optimum techniques to correctly identify *S. aureus* isolates with VISA subpopulations. Valid quantitative antibiotic sensitivity test are: broth dilution, agar dilution, and agar gradient diffusion (Etest; AB-Biodisk). To correctly measure vancomycin susceptibility, CLSI recommend broth microdilution test done in cation-fixed Mueller-Hinton broth using a bacterial inoculum of 0.5 in McFarland scale, and incubating the dilution at 35 ℃ for 24 h[40]. In laboratories that use automated systems or disk diffusion testing, they recommend using a commercial set prepared with brain-heart infusion agar plate with a vancomycin concentration of 6 μg/mL. By these standards, when a *S. aureus* isolate shows a MIC of ⩾ 2 μg/mL (according to the latest MIC classification) it should be confirmed with retesting. If confirmed, this information should be reported as possible VISA (or VRSA if it were ⩾ 16 μg/mL), and depending on the country and institution policies, should be communicated to the hospital's infectious diseases

department, and established health authorities[7].

Sometimes microbiology laboratories will only inform of the MIC cut-off point, and some automated antimicrobial sensitivity tests will not detect *S. aureus* isolates with a MIC of 2 μg/mL or less, therefore complicating the detection of VISA and hVISA strains[40]. Within VSSA isolates, a higher MIC (2 μg/mL) increases the likelihood of detecting the hVISA phenotype[41]. In a recent meta-analysis, prevalence of hVISA was observed to be of 1.67% (14 studies published in different countries between 1997 and 2001). They found a much higher incidence of hVISA within MRSA, and even though some studies were biased because they only included MRSA. They hypothesise MRSA could be more likely to harbour hVISA or VISA, given that MRSA is usually health-care related and hVISA/VISA represent strains that emerge under heavy antibiotic pressure[42,43].

MIC elevation has important consequences for the effectiveness of this antibiotic and therefore has an impact on MRSA bacteraemia mortality. Consequently, it would be reasonable to consider vancomycin as a suboptimal treatment for strains with a vancomycin MIC > 1 μg/mL. The majority of the hospitals and health centres routinely use automated tests to estimate vancomycin MIC. However, these methods aren't always comparable to standardized methods (Etest, broth microdilution), on which the outcome data of most of the studies are based. Automated systems are able to detect only 10% of MRSA isolates with a vancomycin MIC of 2 μg/mL. Given these disparities the reference method (usually broth micro-dilution) must always be used as confirmation[44]. Unfortunately, these results may take days to become available, which could delay adequate specific therapy[45].

MIC "creep"

MIC "creep" is the concept of a surreptitious constant vancomycin MIC elevation in *S. aureus* isolates resulting from various factors such as antibiotic exposure and changes in the *S. aureus* clonal population. The clinical impact of MIC "creep" is yet to be determined, however, a feared consequence could be increased mortality and treatment failure in high vancomycin-MIC *S. aureus* infections, treated with vancomycin[29].

Evidence of this concept is reflected in various studies and epidemiologic analysis that have been carried out in the last ten to fifteen years. For instance, an analysis of 6003 *S. aureus* isolates in Los Angeles-California in 2004, found a trend of increasing vancomycin MIC; the prevalence of *S. aureus* with vancomycin-MIC of 1.0 μg/mL increased from 19.9% in 2000 to 70.4% in 2004, when previously most stood at < 1.0 μg/mL[39]. Similarly, in 241605 *S. aureus* isolates tested by the Surveillance Network Database in the United States of America in 2007, they found 16.2% of them had a MIC of 2 μg/mL[30]. In an evaluation of more than 35000 strains of *S. aureus* isolated during 1998-2003, 4.7% to 7.8% of *S. aureus* isolates had a MIC of 2 μg/mL[46]. Moreover, despite the tapering in CLSI's vancomycin breakpoint to 2 μg/mL, one study demonstrated that 80% of the organisms with MICs of 2 μg/mL were demonstrated to have hVISA phenotypes[47]. Thus, we are witnessing a gradual decrement in vancomycin susceptibility which seems to be greater in settings where the drug is most used[48].

Risk factors for vancomycin MIC elevation

In Lubin's prospective study they analysed predictive factors for an elevated vancomycin MIC in *S. aureus* bacteraemia. In the univariate analysis, various variables were associated with high vancomycin MIC; age > 50 years, the presence of sepsis or shock at the time of culture, a known history of MRSA bacteraemia, recent exposure to vancomycin or daptomycin, and the presence of a prosthetic heart valve or non-tunnelled central line. However, in the final predictive model, only age > 50 years, history of chronic liver disease, recent vancomycin exposure (> 48 h during the previous 7 d), presence of a non-tunnelled central venous catheter at the time of culture, and a history of MRSA bacteraemia were included[45].

A recent study carried out in the United States identified several risk factors for reduced vancomycin susceptibility *S. aureus* infection. A previous history of vancomycin exposure, in the month prior to *S. aureus* isolation (OR = 13) or in the previous 3-6 mo (OR = 2.8), and having any positive culture for MRSA in the previous 2-3 mo were independently related to reduced vancomycin-susceptibility *S. aureus* infections[49].

In a study analysing bacteraemia due to MRSA isolates comparing those manifesting hVISA to those fully vancomycin susceptible, they found association between hVISA and infections with high bacterial load, (*i.e.*, endocarditis), resulting statistically significant. These strains were also associated to longer duration of fever, longer time to clearance of the bacteraemia, length of hospital stay, and failure of vancomycin treatment. Furthermore, they found these strains had frequently been under initial low serum vancomycin levels[50].

Other risk factors observed in previous studies have been admittance to an intensive care unit, female sex, elevated body mass index, recent surgery, and cardiovascular disease[51-53].

Fortunately, VRSA infection continues to be a rare occurrence. In the analysis of the few cases observed, some predisposing risk factors for VRSA infections have been identified. These are; previous enterococcal or MRSA colonization or infection, comorbidities such as diabetes or chronic skin sores and ulcers, and vancomycin exposure. Infection control and antibiotic stewardship are crucial to avoid the emergence of VRSA. However, more studies are needed to better define the specific microbiological and clinical characteristics of these strains[54].

Recently, the first VRSA was detected in Europe, in a Portuguese hospital. In the epidemiological study, the

patient and 53 contacts were screened for *S. aureus* colonization. All strains recovered were characterized by molecular typing methods, by which they observed that VRSA remained confined to the infected foot of the patient and was not detected in any of the close contacts. Only one of the MRSA isolates detected in the screened population was closely related to the VRSA. The VRSA isolated in Portugal belonged to clonal complex (CC) 5, like most of the characterized VRSA strains from other countries. A recent increase in the incidence of lineages belonging to CC5 has been observed in some European countries. This may result in more frequent opportunities for the emergence of VRSA[55].

CLINICAL RELEVANCE OF VANCOMYCIN MIC ELEVATION

Adjusting vancomycin dose to improve AUC/MIC

Vancomycin exhibits concentration-independent and time-dependent killing. Vancomycin efficacy is best measured using the ratio of the 24-h area under the concentration-time curve (AUC_{0-24}) to the MIC ratio (AUC_{0-24}/MIC), in pharmacodynamic parameters. These findings are based on neutropenic murine thigh-infection models[56]. In patients with *S. aureus* pneumonia, treated with vancomycin, it has been observed that attaining an AUC_{0-24}/MIC $\geqslant$ 350 (MIC determined by broth microdilution) is associated with seven times better odds of clinical success. They found shorter time to bacterial elimination when the AUC_{0-24}/MIC attained was $\geqslant$ 400[57,58]. The AUC_{0-24}/MIC concentration obtained with the usual doses administered (1 g/12 h) and with a trough vancomycin concentration of 10 μg/mL, is approximately 400 mg/h per litre. From these results we could assume that the commonly recommended dose is adequate to treat *S. aureus* infections with a vancomycin MIC $\leqslant$ 1 μg/mL but suboptimal when it is > 1 μg/mL.

In the last years, there have been several publications regarding diminished efficacy of vancomycin in those cases with *S. aureus* infection with high vancomycin MIC but within the sensitivity range[38,59,60]. Based on these findings, some studies suggest the ideal aimed vancomycin-dose for best clinical results should be an AUC_{0-24}/MIC ratio $\geqslant$ 400, and therefore targeted trough concentrations should be increased to 15–20 mg/L[61]. However, in a recent study with patients with severe MRSA infections treated with vancomycin in which they adjusted daily dose to reach trough concentrations $\geqslant$ 15 mg/mL, they observed that the cure rate for the cases with vancomycin MIC = 2 μg/mL was still inferior to those with MIC $\leqslant$ 1 μg/mL (62% *vs* 85%; $P = 0.02$)[62].

On the other hand, aggressive dosing strategy could possibly enable targeted vancomycin concentrations in most cases of vancomycin-susceptible *S. aureus* infection. However, this could possibly be unachievable in other clinical settings, such as higher MIC or when limited by vancomycin toxicity. In a recent study, Patel showed that creatinine clearance and vancomycin MIC were inversely related to the probability of achieving adequate AUC_{0-24}/MIC values. He used Monte Carlo simulations to carry out this study. As an example, when administering 1500 mg of intravenous vancomycin every 12 h, target AUC_{0-24}/MIC values were attained in 97% of the cases of vancomycin MIC $\leqslant$ 0.5 mg/L, but they weren't able to reach this target in 38% of the cases with a vancomycin MIC of 2 mg/L[63]. When evaluating vancomycin-susceptible *S. aureus*, VISA, and hVISA in this model, the AUC_{0-24}/MIC ratio required for a static effect was similar for all these organisms. However, the dose required for a 2 $\log^{10}$ kill was 2.5-fold higher for hVISA, compared with VISA. Therefore, the authors concluded that a AUC_{0-24}/MIC ratio of at least 500 was needed to optimize vancomycin pharmacodynamics for hVISA. To attain this AUC_{0-24}/MIC ratio, the needed doses to administer would be extremely high and would involve unacceptable toxicity[64].

In Ghosh's recently published study, they evaluated the utility of previously validated AUC predictions (based on creatinine clearance estimation) and explored the optimal AUC_{0-24}/MIC targets for vancomycin in patients with MRSA bacteraemia. They also investigated whether observed targets are influenced by the sources of the bacteraemia. Treatment failure (persistent bacteraemia, microbiological failure and 30-d all-cause mortality) in their study occurred more frequently in those cases where the AUC_{0-24}/MIC (by broth microdilution) was less than 398 (54% *vs* 23.4%, $P < 0.01$). Other variables associated with treatment failure were chronic lung disease, on-going immunosuppressive treatment, and high-risk sources of bacteraemia (endovascular, pneumonia, complicated intra-abdominal and central nervous system foci). In their study they also observed significant differences between MIC calculated by Etest or microdilution, as previously described. Etest generally yielded MIC results approximately 1-2 dilutions higher than broth microdilution, which could be circumvented by aiming appropriate MIC-method specific AUC_{0-24}/MIC targets. They also observed that bacteraemic source specific AUC_{0-24}/MIC thresholds may offer better outcome in high risk bacteraemia, with lower doses required for low risk source of infection. However, no further studies have yet been carried out to include these findings in current guidelines for their implementation. The controversy of this study relies also in the fact that they found no significant differences in clinical outcome in those cases with high vancomycin MIC (both measured by Etest or broth microdilution). Finally, they conclude that vancomycin trough concentrations are unlikely to accurately reflect AUC_{0-24}/MIC targets and may result in suboptimal outcomes. They suggest AUC estimation based on validated formulas, may allow for individual patient-dose optimisation resulting in increased treat-

ment success when a vancomycin AUC_{0-24}/MIC of ⩾ 398 is achieved[25].

Risk of nephrotoxicity with elevated vancomycin doses

There is limited data suggesting a direct causal relationship between toxicity and specific serum vancomycin concentrations[61]. However, targeting vancomycin dosing for trough concentrations of 15-20 mg/L leads to a greater risk of nephrotoxicity[65], especially in those patients who also receive other nephrotoxic drugs[66]. In fact, a vancomycin trough concentration > 15 mg/L has shown to be an independent predictor of nephrotoxicity[67]. In one the studies proving this association, they compared nephrotoxicity (defined in their study as a 25% decrease in creatinine-clearance rate) developed in 59% of the patients treated with vancomycin that achieved trough serum concentrations of 15 mg/L, whereas in only 30% of those achieving lower trough concentrations (P = 0.0006)[68]. Nephrotoxicity is frequently a limiting factor for patients to receive the optimal doses in MRSA infections, even when adjusted by AUC_{0-24}/MIC, and often forces rotation to other less validated antibiotic schemes.

CONSEQUENCES OF AN ELEVATED VANCOMYCIN MIC IN INFECTIONS BY MRSA AND MSSA

As it has been previously mentioned, glycopeptides, mainly vancomycin has traditionally been the treatment of choice for MRSA infections. However, because of the numerous studies declaring worse outcome in MRSA infections with vancomycin MIC > 1.5 μg/mL, even after adjusting trough concentration to higher thresholds (15-20 mg/L)[63,69], confidence in this treatment option has somewhat declined. This is similarly observed in hetero-resistant strains, observing a loss of bactericidal activity (tolerance) to glycopeptides and more frequent treatment failure in hVISA[70]. Infections where hVISA are isolated have been associated with high-inoculum infections, persistent bacteraemia and metastatic complications, however, up to now, there are controversial results regarding the impact on mortality in these patients[71].

Along these lines, previous studies have declared worse clinical outcomes in *S. aureus* infections, especially bacteraemia, with decreased vancomycin susceptibility (within VSSA ranges)[60,62,69,72]. These results have been reproduced for VISA and hVISA infections, finding bloodstream infections by these strains were more frequently associated to persistent bacteraemia than those that did not show this phenotype[50]. In another study yet, they observed that MRSA bacteraemia was associated with higher mortality when vancomycin was used empirically, on those cases where vancomycin MIC was > 2 μg/mL[60]. But not all publications corroborate these results, generating further controversy[73-75].

A recent meta-analysis which included a total of 22 studies[69] studied the impact of a vancomycin MIC ⩾ 1.5 μg/mL on the clinical outcome of *S. aureus* infections. In this meta-analysis they highlighted an association between higher vancomycin MIC in MRSA infections and poorer outcomes (even mortality), regardless of the source of infection or MIC methodology (OR = 1.64; 95%CI: 1.14-2.73, P = 0.01). They described an increased all-cause 30-d mortality in MRSA bloodstream infections with a vancomycin MIC of 2 μg/mL (determined by Etest), however, no mortality differences were detected in isolates with a MIC of 1 μg/mL and 1.5 μg/mL. Treatment failure, defined as persistent bacteraemia, was also more frequently observed in cases of high vancomycin MIC. After these results have been published, other authors have approached this association with discordant conclusions.

Therefore, despite some contradictory findings, it seems the observation of a higher vancomycin MIC has been repeatedly shown to confer a worse prognosis for MRSA bacteraemia[60,62,69]. This association, however, has been scantily investigated for MSSA strains. The present evidence is scarce, however some studies described a similar association between worse clinical outcome and elevated vancomycin MIC in MSSA bacteraemia, regardless of antibiotic treatment administered (anti-staphylococcal penicillin or vancomycin). In a study of 99 patients with MSSA catheter-related bacteraemia, vancomycin MIC (Etest) ⩾ 1.5 μg/mL was the only independent risk factor for the development of complicated bacteraemia (OR = 22.9; 95%CI: 6.7-78.1), regardless of the initial antibiotic administered[76]. Similarly, another study revealed that mortality increased 2.4-fold in patients with a vancomycin MIC > 1.5 μg/mL, and the choice of antibiotic treatment had no statistical significant effect on 30-d mortality in the multivariable model[72].

S. aureus is one of the main causes of infective endocarditis (IE), being MSSA more frequently found to be responsible for native-valve IE (85% *vs* 15%) compared to MRSA, with a very high morbidity and mortality, that sits around 25%[77-79]. In 2009, a study carried out to analyse the effect of vancomycin MIC on the outcome of MRSA endocarditis, revealed persistent bacteraemia, heart failure and mortality were associated to vancomycin MIC > 1.5 μg/mL[80]. More recently described, higher vancomycin MIC left-sided MSSA endocarditis were more frequently associated with systemic emboli and a higher in-hospital and one-year mortality. In this study, patients with endocarditis by a MSSA strains with a vancomycin MIC ⩾ 1.5 μg/mL (determined by E-test) had 3-fold higher mortality (OR = 3.1; 95%CI: 1.2-8.2)[81].

Evidence that the mechanisms underlying worse clinical outcomes in high-MIC VSSA infections go beyond antibiotic failure was given in a recent multi-centre observational cohort study of 532 *S. aureus* bacteraemic patients[72]. In this study, increasing vancomycin MIC was associated with increased mortality in vancomycin-

Table 2 Treatment recommendations in *Staphylococcus aureus* with reduced vancomycin susceptibility infections[1]

General recommendations	
Removal of indwelling hardware (prosthetic devices, surgical material, intravascular catheter, *etc.*)	
Surgical debridement of infected wounds and abscess drainage	
Follow specific guidelines and local protocols, based on infection site, for treatment duration decisions	
Antibiotic treatment considerations	
Vancomycin	If used aim: AUC_{0-24}/MIC ⩾ 400 or trough blood concentrations of 15-20 mg/L
	Careful monitoring of renal function is imperative
Daptomycin	Bactericidal. Good results with VISA and VRSA endovascular infections
	Consider administration of higher doses (*i.e.*, 10 mg/kg per day) in severe infections and if vancomycin MIC > 2 μg/mL (including VISA)[2]
	Consider synergic combinations (*i.e.*, cloxacillin, aminoglycosides, betalactans, fosfomycin) in infections involving high inoculum (as in IE) and prosthetic devises
	It is inhibited by pulmonary surfactant, therefore should be avoided in SA respiratory or lung infections
	Monitor CK and liver function
Linezolid	Bacteriostatic
	Protein synthesis inhibitor. Inhibits bacterial toxin synthesis
	High tissue bioavailability
	Good results in SSTI and pneumonia (including VAP)
	Oral formulation with similar bioavailability
	Myelotoxicity: Monitor CBC
	Severe interactions with SSRIs and MAOIs, must not be given simultaneously
Tigecycline	Low plasma concentrations. Bacteriostatic. Avoid monotherapy

[1]Treatment recomendations for SA with reduced vancomycin susceptibility usually take methicillin resistance for granted. If the strain were methicillin sensitive, the latter would be the treatment of choice; [2]In VISA and SA with MIC > 2 μg/mL, worse results with lower daptomycin doses have been observed, probably related to cell wall thickness changes in these strains. AUC: Area under the curve; MIC: Minimum inhibitory concentration; VISA: Vancomycin-intermediate *Staphylococcus aureus*; VRSA: Vancomycin-resistant *Staphylococcus aureus*; IE: Infective endocarditis; SA: *Staphylococcus aureus*; CK: Creatinine kinase; SSTI: Skin and soft tissue infections; VAP: Ventilator associated pneumonia; CBC: Complete blood count; SSRI: Selective serotonine reuptake inhibitor; MAOI: Monoamine oxidase inhibitor.

treated patients. Moreover, even in patients with MSSA bacteraemia treated with flucloxacillin, mortality was higher if the vancomycin (Etest) MIC of their isolate was > 1.5 μg /mL, compared with those with lower MIC isolates (26.8% *vs* 12.2%; $P < 0.001$). These results suggest that apart from antibiotic choice, other factors (clinical and microbiological) might be crucial in patient outcome.

Interestingly, despite previous information about poorer prognosis associated to elevated vancomycin MIC, these strains have been found to be associated with a diminished inflammatory response, and therefore less incidence of septic shock. This suggests these strains could have alterations in their pathogenic activity and virulence[60,73]. Peleg studied the pathogenesis of *S. aureus* infections using *Galleria mellonella*. Using both clinical and laboratory strains, they demonstrated that with the evolution of reduced susceptibility to vancomycin, the virulence of *S. aureus* becomes attenuated. The degree to which virulence is attenuated appears to be proportional to the vancomycin MIC[74].

Therefore the arguments linking vancomycin resistance with both reduced bacterial fitness and increased virulence, are yet to be proved, and more studies are needed to determine their clinical significance[6].

Other virulence and prognostic factors in *S. aureus* infections that have taken a leading role are the expression and function of certain genes. The dysfunction of accessory gene regulator (*agr*), has been found to possibly play a key role in MRSA virulence, and seems related to vancomycin resistance. The *agr* locus is in charge of regulating the expression of certain virulence genes and other constitutive genes, required for the maintenance of basic cellular function. The overexpression of *agr* increases the toxin production and reduces the expression of cell surface adhesins[6]. The *agr* locus dysfunction has been associated with reduced vancomycin susceptibility[82], persistent MRSA bacteraemia[58,83], and increased mortality[84], and has been considered a subrogated marker of health-care associated situations. However more studies and investigations are needed to establish the impact of these findings and the possible consequences derived in daily clinical practice (treatment modifications it could imply, invasive procedures to asses, *etc.*).

Overall, there seems to be numerous studies and data indicating that elevated vancomycin MIC in both methicillin resistant and sensitive VSSA, could be a subrogated virulence marker, however, these results are yet controversial, and have not been universally proven. In this realm of controversy, evidence based clinical decisions seem like an arduous and complicated task.

ALTERNATIVE THERAPIES FOR MRSA AND MSSA INFECTIONS WITH REDUCED VANCOMYCIN SUSCEPTIBILITY (TABLE 2)

Clinical approach to S. aureus with > 1.5 vancomycin MIC infections

Up to now, there is no evidence-based unified clinical approach for patients with *S. aureus* infections that have an elevated vancomycin MIC within the sensitivity thresholds (MIC ⩾ 1.5 μg/mL). If the

Table 3 Infection control recommendations for patients colonized or infected by drug-resistant *Staphylococcus aureus* (vancomycin-intermediate *Staphylococcus aureus*, vancomycin-resistant *Staphylococcus aureus*, and methicillin-resistant *Staphylococcus aureus*, Centers for Disease Control and Prevention recommendations[1])

Spread prevention
Isolate patient in a private room
Facilitate gowns and gloves to enter the room
Facilitate mask protection
If risk of aerosol spread consider mask use
Practice hand hygiene with an antibacterial agent (preferably chlorhexidine-based soaps or solutions)
Avoid sharing equipment among patients
Continue isolation until results of tests of nares and infected sites are negative 3 times over 3 wk (including hospital readmission)
Minimize number of staff caring for patient
Educate staff about appropriate precautions and assess compliance
Infection control in nosocomial spread and evaluation
Perform baseline and weekly cultures of hands and nares of healthcare workers in charge of index patient
Consider baseline and weekly cultures for other healthcare workers and persons with extensive contact
Decolonize index patient and healthcare workers with topical mupirocine
Consider avoiding direct patient-contact of colonized healthcare workers until negative culture

[1]Centers for Disease Control and Prevention Healthcare-associated Infections recommendations and guidelines (http://www.cdc.gov/HAI/prevent/prevent_pubs.html).

decision were to use vancomycin, it seems crucial to beware of the different formulas to attain adequate AUC/MIC targets. More studies are needed to consider the contrasting efficacy of other antibiotic treatments in this situation.

The possibility of it being a surrogated virulence marker possibly implies these cases should be up-scored in the severity scale, and this awareness should be maintained for clinical decision-making. Current recommendations include patient isolation and infection control policies, similar to other cases of multi-drug resistant microorganisms infections or colonization (Table 3)[7]. However there are no present studies that define specific indications or clinical algorithms in these cases.

New drugs for MRSA

Daptomycin: Resistance to daptomycin (MIC $\geqslant$ 1 μg/mL) is infrequent and the strains that have shown elevated MIC have been found to have mutations associated with cell membrane structure and cell wall thickness (mprF, yycFG). This has been more frequently observed in VISA strains with a lower sensitivity to glycopeptides[85].

The main concern with the use of daptomycin is the emergence of resistance in the course of treatment. This problem seems to appears more frequently in cases where daptomycin is introduced as rescue treatment after vancomycin has failed or in cases of hVISA or VISA infections[86]. A study carried out in the United States observed a correlation between *S. aureus* strains with reduced vancomycin-susceptibility and the emergence of intra-treatment daptomycin resistance. This was especially found in cases of MRSA infections with vancomycin MIC of 4 μg/mL or greater[87].

Given that daptomycin's mechanism of action is unique, there doesn't seem to be crossed resistance with other antibiotics. Nevertheless, some studies have observed that *S. aureus* with higher vancomycin-MIC (4-16 μg/mL) also show reduced sensitivity to daptomycin[87]. In those strains with vancomycin MIC 4-16 μg/mL, daptomycin MIC was $\geqslant$ 2 μg/mL, and therefore would fall over the sensitive threshold. However, *vanA* resistance to vancomycin does not affect daptomycin sensitivity[86]. In short, reduced vancomycin MIC is a call for awareness and precaution before using daptomycin in *S. aureus* infections. In these cases it would be important to know precise daptomycin MIC (broth micro-dilution or Etest) before starting this treatment.

In order to overcome these difficulties and to increase the bacterial-killing activity, it has been recommended to use higher doses of daptomycin in high risk infections (8-10 mg/kg per day) and up to now there has not been more toxicity associated to these doses in healthy volunteers treated for 14 d[88]. Another possible option that is lately being considered, especially in infections that involve prosthetic materials is the use of daptomycin in combination with other antibiotics. The combination with aminoglycosides and rifampin, has shown to be synergic[89]. Other synergic combinations in experimental models and in preliminary studies with promising results are, cloxacillin[90,91] other betalactams[92-94] and fosfomycin[95]. These options have been mainly studied for MRSA infections and for prosthetic devices associated infections. Future guidelines may contemplate treatment of MRSA infections with borderline vancomycin susceptibility with any of such combinations.

Linezolid: The main disadvantages of using linezolid for high-risk infections that need antibiotic therapy for an extended period of time (*i.e.*, endocarditis) are that it is a bacteriostatic anti-staphylococcal and its myelotoxicity. On the other hand, it offers the advantage of the possibility of oral administration and

has high tissue distribution. In an endocarditis study, linezolid was effective in 4 out of 8 patients (50%) with IE by VISA (MIC 2-4 μg/mL) that had failed with vancomycin[96]. In another retrospective study, 70% (of 22 patients) with MRSA IE that received linezolid because of failure of vancomycin or as sequential oral treatment were cured[97]. Sequential treatment showed 100% cure rate in 8 patients with MRSA IE with early valve surgical replacement (mean 5 d). Linezolid was administered from the fifth day onwards during approximately 3 wk[98]. Therefore, linezolid is an option for selected IE cases, when other treatments fail, or in patients that have intolerance to other treatments.

MRSA resistance to linezolid was first described associated to ribosomal mutations, and recently it has been described related to the appearance of *cfr* (for chloramphenicol-florfenicol resistance gene) gene. This is a plasmid-borne methyltransferase-mediated resistance mechanism that leads to resistance to various antibiotics, as well as linezolid[99,100]. This gene is responsible for the synthesis of a methylase that interferes with 23S rRNA. Hospital outbreaks of linezolid-resistant infections have been associated to these mutations.

Other: There is little clinical experience in the treatment of MRSA severe infections (such as endocarditis or pneumonia) with the "new" glycopeptides such as dalbavancin, oritavancin or telavancin, with tigecycline, or with the new cephalosporins (ceftobiprole or ceftaroline). However the majority of these drugs have shown potential efficacy in experimental models[101-106].

In two recently published trials oritavancin and dalbavancin, showed to be non-inferior to vancomycin and linezolid for the treatment of skin and soft tissue infections (SSTI). These new glycopeptides offer unusual pharmacodynamic and pharmacokinetic properties that allow treating once or twice a week, as has been proved in skin and soft tissue infections, however more studies are needed for other sources of infection[107,108]. Telavancin is approved for the treatment of adult patients with complicated SSTIs and nosocomial pneumonia caused by gram-positive bacteria, including MRSA, when no other options are available. Up to now, the use of telavancin is restricted to MRSA infections with a vancomycin MIC $\geqslant$ 1 μg/mL, hVISA infections, lack of response to vancomycin treatment or patients who do not tolerate other antistaphylococcal antibiotics[109].

Tigecycline is a semisynthetic drug derived of minocycline. Being a broad spectrum antibiotic, it has anti gram-positive and gram-negative activity[110]. Drawbacks to the use of tigecycline in bloodstream infections come from both, intrinsic drug characteristics and clinical experience. It is a bacteriostatic antibiotic and it reaches high tissue concentration but low concentration in plasma. Moreover, in previous studies it has been associated to worse prognosis and higher mortality rates in patients with severe infections. Consequently, tigecycline is not normally recommended as a first line antibiotic for bloodstream or severe MRSA infections[111,112].

Ceftaroline-fosamil is a cephalosporin with anti-MRSA activity[113]. In two clinical trials comparing ceftaroline with vancomycin plus aztreonam for SSTIs, they found treatments were comparable[114]. There is still little experience in the use of ceftaroline in other sources of MRSA infections.

Ceftobiprole is a new cephalosporin, that shows a broad-spectrum and strong bactericidal activity even for MRSA[115]. Ceftobiprole has high affinity for PBP2a (main PBP responsible for methicillin resistance), and is also stable to class A penicillinases, thence its good anti-MRSA activity[115]. In an animal MRSA endocarditis model, ceftobiprole was found superior to vancomycin, daptomycin, and linezolid[116].

Combination therapies

Rifampin and gentamycin are the two antibiotics that have most frequently been associated to vancomycin. The use of rifampin is based on its activity against *S. aureus* in stationary phase. However this synergy has not been proved *in vitro*[117,118] and the clinical benefits of adding rifampin to vancomycin in the treatment of MRSA IE hasn't been proved either[119]. The association of vancomycin and an aminoglycoside has been found synergic[120] and is therefore contemplated in patients with persistent bacteraemia. However this association hasn't proved a lower mortality rate in IE, and it has shown increased nephrotoxicity[121], so it probably shouldn't be held as a first option treatment.

The combination of vancomycin and linezolid, both *in vitro* and *in vivo*, is indifferent or possibly antagonistic[122]. There are experimental studies[123] and a scarce clinical experience[124] that showed that the combination of vancomycin and quinupristin-dalfopristin was synergic, safe and useful for the treatment of 5 patients with severe MRSA infections.

There is scarce evidence about possible antibiotic combinations with linezolid, and results are contradictory. *In vitro* studies have shown decreased antibiotic activity of both gentamycin and vancomycin when associated to linezolid[125]. On the other hand, in animal models of IE they observed advantages in the combination of linezolid and gentamycin *vs* only linezolid[126]. Synergic combinations of linezolid with ertapenem and imipenem have also been communicated, both *in vitro*, and in experimental endocarditis models. However this association only is observed if the carbapenem is given in sub-inhibitory doses, whereas therapeutic doses decreases linezolid's antibiotic activity[127].

Daptomycin at a dose of 10 mg/kg per day (and perhaps higher) may be more effective than the currently approved 6 mg/kg per day dose for severe *S. aureus* infections caused by non-susceptible strains (*i.e.*, those with MICs of > 1 μg/mL)[128]. In an experimental

animal aortic valve MRSA endocarditis model, combinations of daptomycin with an aminoglycoside or rifampin didn't show synergy[129].

While fosfomycin is Food and Drug Administration approved only for the treatment of uncomplicated urinary tract infections, it has demonstrated good antimicrobial activity against a broad spectrum of pathogens, including MSSA and MRSA[130]. Fosfomycin, which acts by inhibition of an early step in cell wall synthesis, has been used successfully in combination with beta-lactams to treat severe staphylococcal infections[131]. It also shows *in vitro* synergy when combined with daptomycin[132]. Three cases have been recently published where they observe that the *in vitro* combination of high doses of daptomycin plus fosfomycin can be effective in the treatment of both native- and prosthetic-valve endocarditis caused by MSSA or MRSA[133].

CONCLUSION

MRSA proves to be a persistently lurking microorganism underlying both community and healthcare associated infection. The emergence of increasing vancomycin resistance patterns and the different consequences derived have created a new area of uncertainty in the clinical and therapeutic approach to these infections. More studies and trials are needed in order to better define these issues.

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MINIREVIEWS

Origin of *de novo* daptomycin non susceptible enterococci

Theodoros Kelesidis

Theodoros Kelesidis, Department of Medicine, Division of Infectious Diseases, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, United States
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Correspondence to: Theodoros Kelesidis, MD, PhD, Department of Medicine, Division of Infectious Diseases, David Geffen School of Medicine at UCLA, 10833 Le Conte Ave, CHS 37-121, Los Angeles, CA 90095, United States. tkelesidis@mednet.ucla.edu
Telephone: +1-310-8257225
Fax: +1-310-2080140
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Abstract

The emergence of daptomycin non-susceptible enterococci (DNSE) poses both treatment and infection control challenges. Clinicians should be vigilant that DNSE may be isolated from patients with or without (*de novo* DNSE) prior use of daptomycin. Recent epidemiological data suggest the presence of a community reservoir for DNSE which may be associated with environmental, foodborne and agricultural exposures. The mechanisms of nonsusceptibility to daptomycin have not been well characterized and may not parallel those for *Staphylococcus aureus*. The identification of daptomycin resistance genes in anaerobes, in farm animals and in an ecosystem that has been isolated for million years, suggest that the environmental reservoir for *de novo* DNSE may be larger than previously thought. Herein, the limited available scientific evidence regarding the possible origin of *de novo* DNSE is discussed. The current existing evidence is not sufficient to draw firm conclusions on the origin of DNSE. Further studies to determine the mechanisms of *de novo* daptomycin nonsusceptibility among enterococci are needed.

Key words: Daptomycin non-susceptible enterococci; Antimicrobial resistance; Environmental reservoir

Core tip: Daptomycin non-susceptible enterococci (DNSE) is an emerging clinical problem and may be isolated from patients with or without (*de novo* DNSE) prior exposure to daptomycin. Recent epidemiological data suggest the presence of a community reservoir for DNSE which may be associated with environmental, foodborne and agricultural exposures and may be larger than previously thought. Herein, the limited available scientific evidence regarding the possible origin of *de novo* DNSE is discussed. Further studies to determine the mechanisms of *de novo* daptomycin nonsusceptibility among enterococci are needed.

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INTRODUCTION

Antibiotic resistance is a major threat to human health[1]. Multidrug-resistant organisms such as vancomycin-resistant enterococci (VRE) may increase morbidity and mortality[1]. Daptomycin has bactericidal activity against VRE. However, daptomycin non-susceptible enterococci

(DNSE) are difficult to treat and clinicians often have limited treatment options[2]. Enterococci with daptomycin MIC > 4 μg/mL are non-susceptible, according to the Clinical Laboratory Standards Institute[3] and the Food and Drug Administration[4]. Although most DNSE isolates develop after daptomycin therapy they have also emerged in subjects with no prior use of daptomycin[5]. Elucidating the origin of *de novo* DNSE infections may help us understand mechanisms of daptomycin non-susceptibility. Herein, the available scientific evidence regarding the possible origin of *de novo* DNSE is reviewed.

OVERALL PREVALENCE OF DNSE IS LOW

Despite initial *in vitro* studies that emergence of DNSE is rare[3,6-10], recent studies suggest that DNSE is an emerging infection[2]. In large surveys of clinical isolates less than 0.6% of *Enterococcus faecalis* (*E. faecalis)* or *E. faecium* isolates were DNSE[11-15]. However, there is lack of data on daptomycin non-susceptible enterococcus isolates from international and national programs[2]. In a recent literature review, DNSE were present in 0.6% of all enterococci isolates (range 0%-19.1%)[2] and out of 150 DNSE isolates, 93.3% were vancomycin resistant enterococci (VRE), 6.0% were vancomycin susceptible enterococci (VSE), 88% were *E. faecium* and 8.7% were *E. faecalis*[2]. Most DNSE isolates were reported in Asia (40.3%) and in Europe (34%) while 26% of isolates were reported in North America[2]. Reporting bias, use of different susceptibility testing method among clinical microbiology laboratories such as MicroScan and presence of clones may overestimate the detection of DNSE[16-21]. Thus, the overall prevalence of DNSE was low.

MECHANISMS OF EMERGENCE OF DAPTOMYCIN RESISTANCE IN ENTEROCOCCI ARE COMPLEX

The mechanisms for daptomycin nonsusceptibility in enterococci are different than in staphylococci and are poorly-understood[22-30]. Whole-genome sequencing of DNSE[30-34] suggest that few genetic mutations may be adequate to induce daptomycin non-susceptibility. Compared to their susceptible counterparts DNSE isolates have mutations in stress response regulators (such as the LiaFSR, yycG and YybT regulatory systems)[29-39], phospholipid composition regulators [such as cardiolipin synthase (Cls), glycerophosphoryl diester phosphodiesterase (GdpD), cyclopropane fatty acid synthase (Cfa)][27-33,40], and phenotypic changes such as reduced cell membrane fluidity[28,30,31,41] and increased septation (*via* Ezr A)[30,31].

DNSE may develop without prior use of daptomycin

Spontaneous emergence of daptomycin non susceptibility *in vitro* is rare[24]. Although DNSE usually emerge in the setting of daptomycin therapy[2] DNSE have also been identified in subjects without prior use of daptomycin[5] and daptomycin use may not be a risk factor for DNSE in a case control study[42]. The risk factors related to emergence of *de novo* DNSE remain unclear.

FACTORS THAT ARE ASSOCIATED WITH DEVELOPMENT OF DNSE

Host factors related to isolation of DNSE

In a review of DNSE isolates, the source patients were 54.6 years on an average and 62.5% of them were female[2]. Factors that may contribute to emergence of DNSE include a source of DNSE infection such as abscess[2], an intra-abdominal pathological process, recent surgery, a lengthy exposure to daptomycin[43,44], immunosuppression and pharmacokinetics[43] and suboptimal drug levels among patients with end stage renal disease[45-47]. Observations from a case report suggested that chronic severe hypocalcemia in one patient may have contributed to the even lower calcium levels at the nidus of DNSE infection (abscesses)[32], which may precipitate a loss of daptomycin activity[48]. Thus, DNSE may occur in the context of the above disorders and only few mutations may occur in DNSE[32].

Antimicrobial exposure may also be a risk factor for emergence of DNSE

Recent case controls studies with DNSE isolates have identified that many risk factors for emergence of VRE, including recent antimicrobial exposure, and increased hospitalization, were also present in the majority of DNSE cases[49]. Recent use of vancomycin, cephalosporins, or antibiotics active against anaerobes is associated with isolation of both VRE and DNSE[49]. VRE often causes colonize the colon[50,51] and vancomycin resistant[52] and daptomycin resistant gut anaerobes have been identified[53]. Resistance to vancomycin in gram-positive bacteria did not affect daptomycin activity[54]. Finally, multiple comorbidities, immunosuppression, and prior exposures to antimicrobials such as metronidazole and cephalosporins were independently associated with the isolation of DNSE (VRE) in a recent study[42].

Exposure to daptomycin has may contribute to emergence of DNSE especially in the setting of end stage renal disease

Although previous studies suggest that daptomycin resistance develops during treatment, MICs for daptomycin were often not reported[2]. In a review of DNSE isolates, the dose and duration of daptomycin that was administered prior to isolation of DNSE[2]. In one study, daptomycin-exposed DNSE patients received an

average of 44.9 d of daptomycin therapy[49]. Patients with end stage renal disease have lower C_{max} for daptomycin compared to healthy subjects[55] and the concentrations of daptomycin used in these patients may be relatively low[55-58]. Thus, more research should determine the optimal dosage and frequency of daptomycin administration in patients with end stage renal disease[43,44] since enterococci may become DNSE rapidly[32].

FACTORS RELATED TO ISOLATION OF *DE NOVO* DNSE

Limited data suggest that host factors are not known to be related to isolation of de novo DNSE

We found no significant differences in terms of age, sex and underlying immunosuppressive illnesses between patients with *de novo* DNSE infections and DNSE infections following exposure to daptomycin[49].

Environmental factors related to emergence of de novo DNSE

In our series, 45% of patients with DNSE had no prior use of daptomycin and clonally-related DNSE were isolated in patients with no prior hospitalization[49] suggesting an environmental reservoir of DNSE[5]. Shorter duration of hospitalization, less frequent exposure to antimicrobials associated with isolation of VRE, were associated with *de novo* DNSE infection[49] but since DNSE may persist for years[59], nosocomial acquisition of DNSE is possible. Factors that may contribute to formation of an environmental reservoir of DNSE include exchange of genetic material between enterococci, soil bacteria and bacteria of animal origin, foodborne origin of DNSE and agricultural exposures of humans to DNSE.

Transfer of genes that determine antimicrobial resistance between soil bacteria and DNSE may contribute to emergence of de novo DNSE

Daptomycin resistance genes were found in bacteria from an ancient ecosystem[60]. Soil actinomycetes may inactivate daptomycin[6,61] and we have also identified found mutations in DNSE isolates in genes that are also present in soil bacteria[31]. Soil bacteria and enterococci may exchange genetic material[62]. However in another study, mechanisms of inactivation of daptomycin found in soil bacteria were not identified in DNSE *E. faecium*[22]. Thus, it remained to be elucidated whether the interplay between soil bacteria and enterococci may contribute to emergence of DNSE.

Bacteria in animals may mediate acquired daptomycin resistance in enterococci

Humans and animals may exchange daptomycin resistance genes and this may lead to emergence of *de novo* DNSE[63]. The gut of humans and most animals harbors enterococci and VRE can spread from farm animals[64,65]. Enterococci of animal origin may transfer antimicrobial resistance genes to other enterococci[66]. Recombination between repetitive nucleotide sequences[30] that may encode resistance cassettes in enterococci[62,64,65] may contribute to emergence of DNSE. Finally, we also found similar nucleotide mutations in genes that are common between DNSE and bacteria found in poultry[31,67-69].

Limited data suggest that DNSE infections in humans may be foodborne

DNSE may have passed to humans *via* ingestion of meat[5]. Up to 25% of enterococci isolated from beef were DNSE[65]. Daptomycin resistant Enterococci were recently identified in cows[70]. *E. faecalis* may harbor resistance genes and can be passed to humans through meat consumption[71]. Poultry might be a source for *E. faecalis* infections[72] and may harbor *E. gallinarum*[73] which may also be daptomycin non-susceptible[49]. Similarly, all three *de novo* urine DNSE isolates, were *E. faecalis,* may cause zoonosis[74]. In our study 4/9 (44.4%) subjects with *de novo* DNSE infections reported consumption of beef[5]. Thus, it remains to be shown whether DNSE may be foodborne pathogenes[5,65].

Limited data from epidemiological studies and case series suggest that DNSE may have a zoonotic potential

Humans who are exposed to farm animals may be at risk increase to be colonized with multidrug resistant bacteria[75]. We found that in contrast to patients with daptomycin-exposed DNSE, the majority (78%) of *de novo* DNSE infections lived in areas with increased prevalence of agricultural exposures[76]. In our study of *de novo* DNSE infections 33.3% of patients had prior exposure to farm animals[5]. Thus, further epidemiological studies need to confirm if it is possible that exposure of humans to farm animals may increase the risk for isolation of DNSE[63].

Limited data from observational studies suggest that transfer of genes that determine antimicrobial resistance between anaerobes and DNSE may contribute to emergence of de novo DNSE

Enterococci and anaerobes are gastrointestinal tract flora in humans and may exchange antibiotic resistance genes[77,78]. Mutations in phospholipid biosynthesis and lac operon expression exist in facultative anaerobic[79] and anaerobic bacteria[80] may also lead to emergence of DNSE[30,34]. In addition, the use of antibiotics with activity against anaerobes may increase the spread of VRE and DNSE[81] while recent use of metronidazole may be a risk factor for emergence of DNSE[42]. Use of prior antibiotics with activity against anaerobes was found less in patients with *de novo* DNSE compared to daptomycin-exposed patients with DNSE infection[49]. Finally, daptomycin nonsusceptibility has been

described in anaerobes[53]. Thus, further studies need to confirm that the cross talk among anaerobic bacteria and enterococci may contribute to dissemination of DNSE[82].

CONCLUSION

Treatment of DNSE infections is a challenge for clinicians. Daptomycin non-susceptible enterococcal strains may develop after exposure to daptomycin. Since DNSE are usually isolated from patients with many comorbidities such as immunocompromised and end stage renal disease patients, strict infection control and prudent use of daptomycin are needed for these patients to limit the emergence and spread of DNSE.

However, DNSE may emerge without prior use of daptomycin. Recent epidemiological data suggest the presence of a community reservoir for DNSE which may be associated with environmental, foodborne and agricultural exposures. The mechanisms of development of daptomycin resistance remain unclear. The identification of daptomycin resistance genes in an ancient ecosystem[60], in anaerobes[53] and in farm animals[70] suggest that the environmental reservoir for *de novo* DNSE may be larger than previously thought. In most of the studies with reported DNSE isolates complete medical records were not reviewed and interview of patients was not performed and thus potentially relevant occupational or dietary exposures among patients with DNSE were not identified. Epidemiological investigations focused on environmental exposures in the community may help elucidate the origin of *de novo* DNSE. Further studies to identify the mechanisms of *de novo* daptomycin nonsusceptibility in enterococci are needed.

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MINIREVIEWS

Surface adhesion and host response as pathogenicity factors of *Neisseria meningitidis*

Jose Uberos, M Molina-Oya, S Martinez-Serrano, L Fernández-López

Jose Uberos, M Molina-Oya, S Martinez-Serrano, L Fernández-López, Department of Paediatrics, School of Medicine, University of Granada, 18012 Granada, Spain
Author contributions: All authors contributed to this manuscript.

Correspondence to: Dr. Jose Uberos, Department of Paediatrics, School of Medicine, University of Granada, Avda. de Madrid s/n, 18012 Granada, Spain. joseuberos@telefonica.net
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Abstract

Neisseria meningitidis (*N. meningitidis*) is an exclusively human pathogen that has been identified in 10%-35% of the adult population and in 5.9% of the child population. Despite the high prevalence of carriers of *N. meningitidis*, it only occasionally causes meningococcal disease in the context of endemic disease, in certain geographic areas or in isolated epidemic outbreaks. After the *N. meningitidis* genome is described, progress has been made toward understanding the pathogenic mechanisms of the bacteria, although some aspects concerning its interaction with the environment and the host remain unclear. Some studies have reported that oxidative stress in the environment can modify the surface characteristics of *N. meningitidis*, increasing its adhesive properties and favouring an asymptomatic carrier state. The antigenic structure of *N. meningitidis* can be modified by its importing genetic material from other bacteria in its ecological niche. Some structures of lipopolysaccharides help it to evade the immune response, and these are observed more frequently in *N. meningitidis* isolated from blood than in healthy nasopharyngeal carriers. There is evidence that pili and capsule are downregulated upon contact with target cells. This paper reviews current knowledge on host-environment-bacteria mechanisms and interactions, with the aim of contributing to our understanding of the pathogenic mechanisms of *N. meningitidis*.

Key words: Bacterial adhesion; *Neisseria meningitidis*; Virulence

Core tip: After the *Neisseria meningitidis* (*N. meningitidis*) genome is described, progress has been made toward understanding the pathogenic mechanisms of the bacteria, although some aspects concerning its interaction with the environment and the host remain unclear. This paper reviews current knowledge on host-environment-bacteria mechanisms and interactions, with the aim of contributing to our understanding of the pathogenic mechanisms of *N. meningitidis*.

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BACTERIAL ADHESION AND PATHOGENICITY

The ability of bacteria to attach and grow on almost

any surface has been known for decades. The importance of adhesion in the colonisation of specific substrates, and its role in the pathogenesis of bacterial infections and in the maintenance of the carrier state has been studied widely in recent years[1]. *Neisseria meningitidis* (*N. meningitidis)* is only found in humans, suggesting that its ability to cause disease is likely an casual side effect of its life cycle. Globally, the carrier rate of *N. meningitidis* ranges from 10%-35% among healthy adults[2]; the mean carrier rate in children is 5.9%, peaking at 10.3% in children aged under 3 years[3]. This situation has been associated with the genetic characteristics of the circulating strains of *N. meningitidis*, the immune pressure exerted by vaccination programmes and the hygienic and social conditions within a community. Compared with the rates of colonisation, meningococcal disease is less common, its development being affected by interacting factors such as the virulence of the bacterium, host defence mechanisms, the age of the host and the history of previous viral infections[2]. The best-defined virulence factor of *N. meningitidis* is its polysaccharide capsule that indicates its serogroup. Although 13 serogroups of *N. meningitidis* have been described (A, B, C, D, 29E, H, I, K, L, Y, W-135, X and Z), invasive meningococcal disease is most frequently caused by serogroups A, B, C, Y and W-135, to which 10% mortality has been attributed.

The adhesion of bacteria to epithelial surfaces is an initial step in the colonisation of microbial habitats, and it ensures the survival of *N. meningitidis* in its ecological niche[4]. Adherence can be defined as a phenomenon resulting from the interaction between two surfaces, with the participation of physical, chemical and biological factors, with contact between the bacterium and the cell being necessary for adherence to take place. The adhesion occurs in several steps: (1) *N. meningitidis* attaches to the surface of target cells to form small colonies. This step is essentially a pilus-mediated process; and (2) after *N. meningitidis* has been attached, it comes into close contact with surface of the target cells (intimate adhesion). The adhesive interaction is present both in commensal bacteria and in pathogens and so for *N. meningitidis* to adhere, it has developed proprietary adhesive mechanisms that allow it to compete with flora of the same ecological niche[5]. Meningococcal pili are of type Ⅳ and are composed of pilin subunits that are encoded by *pilE* gene. Other homologous proteins, PilC1 and PilC2, are also involved in pilus assembly and adhesion. PilC1-containing may involve interaction with CD46, a human trans-membrane glycoprotein involved in complement regulation. The expression of *pilC1* is induced following the contact of *N. meningitidis* with viable target cells. Both pili and capsule are downregulated upon contact with target cells. This downregulation seems to be associated with intimate adhesion of *N. meningitidis* to target cells[5,6].

The "adhesion process" can be defined in terms of the adhesion affinity of bacteria to epithelial surfaces, as has been described in the Michaelis-Menten equations. The maximum point of adhesion (and affinity) can be determined graphically by the Lineawever-Burk equations, using simple experimental models[7]. This first phase would be a reversible process in which Van der Waals and electrostatic forces are responsible for a wide range of interactions, including chemical bonding, dipolar interaction and hydrophobicity. Surface molecules as *N*-acetylneuraminic acid may alter the initial adhesion strength, reducing electrostatic repulsive forces or increasing attractive ones[8]. The cell surfaces of both prokaryotic and eukaryotic cells are negatively charged. Electrostatic repulsive forces between the cell and the bacteria can be overcome by long and short-range attractive forces, and so the specific binding of fimbriae with cell surface receptors must overcome the repulsive forces between the two surfaces. According to Smyth *et al*[9], the reduction of the surface bacteria potential by the intervention of hydrophobic adhesins probably facilitates adhesion, with the hydrophobic forces being a first step in the interaction of the organism with mucosal surfaces. Surface hydrophobicity is a non-specific adhesion factor which is important to the adhesion and growth of microorganisms on epithelial surfaces. The hydrophobic-hydrophilic environment of the bacterial surface is modulated by hydrophobic or hydrophilic agents (increasing or reducing hydrophobicity, respectively), which may co-exist on the surface of the outer membrane[5]. Generally, the strains that formed biofilms show high-level cell surface hydrophobicity. Many studies have examined the contribution of surface hydrophobicity to bacterial adhesion, with particular attention to *Salmonella*[10,11], *Escherichia coli* (*E. coli*), *N. gonorrhoeae*[12,13] and *N. meningitidis*[14,15].

The type Ⅳ pili of *N. meningitidis* are crucial determinants of the adhesion of these pathogens to epithelial and endothelial cells[16]. Under natural conditions, pili are the only means by which encapsulated *N. meningitidis* can adhere to human mucosal surfaces[17,18].

SURFACE MODULATION AND INTERACTION WITH THE HOST

When *N. meningitidis* adheres to epithelial cells, it becomes resistant to the bactericidal effect of the antimicrobial peptide LL-37, which is the first line of defence of innate immunity. The decreased binding of LL-37 to the adhered bacteria can result from its degradation by proteases released at the site of infection. Furthermore, *N. meningitidis* induces the formation in the nasopharynx of cholesterol-rich membrane microdomains, which are essential to the antimicrobial resistance induced by bacterial adhesion[2].

To avoid immune detection, the surface components of *N. meningitidis* may be modified. The structural and antigenic modification of surface molecules can

involve changes in gene alleles. Studies have reported the import of genetic material from other bacteria or *via* intragenomic recombination[2]. *N. meningitidis* may be encapsulated or unencapsulated. *N. meningitidis* isolated from blood or cerebrospinal fluid is invariably encapsulated. The existence of the capsule enables it to withstand the effects of antibodies and complements and to resist serum opsonic activity. Some lipopolysaccharide structures help *N. meningitidis* to evade the immune response, and are more frequently observed in *N. meningitidis* isolated from blood than in healthy nasopharyngeal carriers. Both the capsule and some lipopolysaccharide immunotypes (L_1, L_8 and L_{10}) of *N. meningitidis* may influence bacterial adhesion and invasive capacity. It has been found the inhibitory role of capsule in biofilm formation[14]. The capsule genes are located in a single chromosomal locus (cps) divided into three regions. The capsular polysaccharides B, C, W-135 and Y contain sialic acid, which contributes to make the lipopolysaccharides of the capsule less visible to the immune system, since sialic acid is a common component of the host cell surfaces. Moreover, the serogroup B capsule contains a homopolymer that is structurally identical to the neural adhesion molecule, which is responsible for the poor immune response generated by serogroup B in humans. However, the genetic similarities of the loci of serogroups B, C, W and Y (not A) favour the horizontal exchange of fragments of the capsule between different serogroups[2].

N. meningitidis expresses and secretes various surface molecules that bind to epithelial molecules, and some of these proteins include lactoferrin and the proteins bound to the transferrin that enable the meningococcus to acquire iron from the environment. Iron is a crucial element for bacterial growth in the surface colonisation stage and during the production of disease[19,20], although some adherent properties, such as hydrophobicity and adherence to inert surfaces like nitrocellulose remain unchanged after incubation in culture media supplemented with Fe[21]. Other authors have described nine porin complexes formed by different combinations of the meningococcal porin protein (Por) A, PorB and RmpM proteins[22]. *N. meningitidis* expresses two types of outer membrane proteins (Opa and Opc) which give an opaque appearance to colonies in agar. Opa and Opc are of a similar size (27-31 kDa). Most Opa molecules recognise one or more members of the family of carcinoembryonic antigen-related cell adhesion molecules (CEACAM). The CEACAM1 receptor is found in epithelial and endothelial cells, while other family members such as CEACAM3 and CEACAM6 are expressed in neutrophils. CEACAM receptor density in the epithelial cells is modulated by the secretion of inflammatory cytokines, such that a high expression of CEACAM receptors takes place in response to inflammation, which could influence the development of meningococcal disease. Furthermore, some Opa proteins can interact with the heparan sulphate proteoglycan that is present in most epithelial cells[2,23].

Over the past 50 years, our understanding of the importance of serogroup B (MenB) disease *per se*, the social impact of fear caused by the devastating effects of the disease. The difficulty of inducing an effective immune response against the MenB capsular polysaccharide has lead to the search in vaccines for this serogroup based on outer membrane proteins (OMP). Public health interventions in Cuba, Norway and New Zealand have demonstrated that these protein-based vaccines can prevent MenB disease.

By combining a pangenome analysis with an extensive experimental validation to identify new potential vaccine candidates, genes coding for antigens likely to be exposed on the surface of the MenB were selected after a multistep comparative analysis of entire Neisseria genomes. Again, in the quest for vaccine candidates are successfully identified a significant number of new genes. Recent studies with meningococcal membrane proteins have centered on conserved antigens in order to obtain a universal vaccine that confers protection against a broad range of strains. There are several recent reports about the use of conserved minor OMP from *N. meningitidis* as immunogens[24].

The classical bioinformatic approaches, in combination with proteomic data, conventional protein purification and immunological evaluation are powerful tools for the identification of novel meningococcal antigens and open reading frames and potential vaccine components[25].

We now know that OMP based vaccines are most effective when are used against epidemics due to a homologous or clonal strain carrying the same PorA as that present in the vaccine. When used against endemic disease or outbreaks due to a number of different strains (heterologous epidemiologic situations), the level of effectiveness will generally be too low to rely on the effects of a conventional OMP vaccine alone for protection.

The general strategy in the Pajon *et al*[25] study, was to maximize the chance of identifying bacterial surface components by selecting not only proteins predicted by protein localization algorithms in outer membrane components of gram-negative bacteria, but also those predicte as periplasmic or inner membrane proteins. However, we must stress that while the most attention in the development of meningococcal vaccines has been devoted to major OMPs. The impact of conserved protein components in the induction of a significant immune response, and their potential as adjuvants, it must not be overlooked. The success in expressing all cloned genes came from the use of a highly optimized expression/purification platform designed precisely for this scenario, but also from the stringent selection procedure of potential vaccine candidates. Finally, five proteins are capable of inducing a functional antibody response *vs N. meningitidis* strain CU385: NMB0606 a potential YajC orthologue, NMB0928 the

neisserial NlpB (BamC), NMB0873 a LolB orthologue, NMB1163 a protein belonging to a curli-like assembly machinery, and NMB0938 (a neisserial specific antigen) with evidence of positive selection appreciated for NMB0928[25].

The new set of vaccine candidates and the novel proposed functions will open a new wave of research in the search for the elusive neisserial vaccine. The key limitation of conventional wild-type outer membrane vesicle (wtOMV) vaccines is their lack of broad protective activity against the large diversity of MenB strains circulating globally. The experience with wtOMV vaccines also provide important information for the next generation of MenB vaccines designed to give more comprehensive protection against multiple strains.

BACTERIAL ADHESION AND OXIDATIVE STRESS

It has been shown that the *N. meningitidis* loci involved in defence against oxidative stress are also involved in biofilm formation and contribute to the colonisation of epithelial surfaces[26]. Incubation of *N. meningitidis in vitro* with antioxidant molecules increases their adherence to inert surfaces and therefore the ability to generate biofilm, and at the same time increases their surface hydrophobicity[14,21]. Similar observations regarding adherence to nitrocellulose have been demonstrated in *E. coli*[27]. These observations are an example of how environmental conditions can modulate in *N. meningitidis* the expression of molecules to make it more virulent or more adherent. *In vivo,* plasma antioxidant levels are lower in children who are asymptomatic carriers of *N. meningitidis*[3]. We have analysed the association between total antioxidant capacity in plasma and the carrier state of *N. meningitidis*. In the carrier state, the odds ratio for this association (total antioxidant capacity in plasma < 0.25) was 8.44 (95%CI: 1.5-48.9). These observations are in the line with Jamet *et al*[26], who reported that the activation in *N. meningitidis* of genes involved in defence against oxidative stress (lower levels of plasma antioxidants) favours the adhesion of the bacteria and nasopharyngeal carrier status. Other studies have shown that oxidative stress can be induced experimentally with cysteine depletion, can trigger growth arrest and release of outer membrane vesicles (sOMV). Outer membrane vesicles contain immunogenic proteins and contribute to *in vivo* survival and virulence of bacterial pathogens[28].

BACTERIAL ADHESION AND VIRULENCE

Virulence, defined as the degree of aggressiveness of a pathogen, is a highly variable condition. The degree of virulence fluctuates according to the conditions in which microorganisms and their genetic makeup are located. In general, a pathogen becomes less virulent on passing from a natural environment to an artificial culture medium; in these circumstances, it is said to be attenuated, and the same effect can be observed in unfavourable environmental conditions. The virulence of a microorganism can be reduced, either by the use of certain culture media, or by exploiting its successive passage through animals. Numerous studies support this; thus, Horská *et al*[29] have reported that three different bacterial strains of *Pseudomonas* are capable of changing the surface charge and their hydrophobicity. By contrast, an attenuated microorganism can acquire greater virulence by its prior passage through certain animal species; specifically, pneumococcal virulence is enhanced by its passage through mice[30]. *N. meningitidis* requires iron, and in the absence of iron alters its gene expression to increase iron acquisition[19,31]. When *N. meningitidis*, has grown in an iron-restricted environment, it synthesises new outer membrane proteins, which are necessary for its survival. Some of these proteins Tbp A and Tbp B are examples of meningococcal surface antigens regulated by iron, which are not expressed after culture in common laboratory media[32]. TbpA was found to possess a similar architecture to the siderophore and is highly immunogenic, allowing for prediction of potentially important ligand-binding epitopes[33].

The passage of microorganisms through the epithelial layer is not a passive phenomenon (Figure 1). Microorganisms, after overcoming the first hurdle, consisting of the surface epithelium, are exposed to various host defence mechanisms, of which the most important is the inflammatory response. In the course of this response, the blood vessels dilate, thereby increasing their permeability and allowing various serum factors (immunoglobulins and other proteins) to come into contact with the infectious agent. Moreover, the activation of fibrinogen to fibrin delays the diffusion of the microorganisms.

In general, type-1 somatic fimbriae are encoded by chromosomal genes and are found both in commensal bacteria and in pathogenic strains of *E. coli*. The adhesion factors that are most frequently associated with pathogenicity are usually coded by plasmids[34], although this may take place chromosomally. The bacterial surface appendages related to association functions are generally termed "fimbriae", with the term "pili" being reserved for cases in which their presence is related to the exchange of genetic material among microorganisms. The pili of *N. meningitidis* are 6 nm in diameter and extend several micrometres into the surface of the bacterium. They are, therefore, sexual or conjunctive fimbriae. The genes for the bacterial adhesion factors that have been most thoroughly studied, such as K-88, K-99 and CFA/I, are located in plasmids. Of these, the genes for factor K-88 are known to have a total length of 75-135 Kb, and are frequently associated with genes for the fermentation of raffinose. The gene for the CFA/I factor has a size

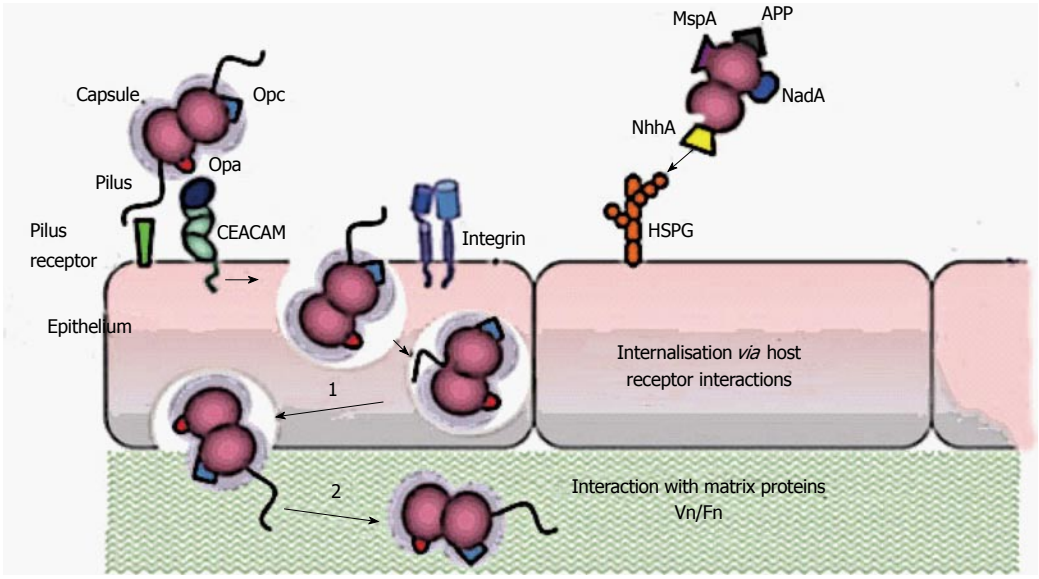

Figure 1 Schematic representation of the interaction mechanisms of *Neisseria meningitidis* with cellular receptors. The first adherence phase would be a reversible process in which Van der Waals and electrostatic forces are responsible for a wide range of interactions, including chemical bonding. Finally we added a summary at the endding, dipolar interaction and hydrophobicity. Pili extending beyond the capsule are considered to mediate the primary interaction with epithelial cells. Opa proteins may bind to carcinoembryonic antigen-related cell-adhesion molecule (CEACAMs) and heparan sulphate proteoglycan (HSPGs), and Opc proteins can interact with HSPGs and, *via* vitronectin and fibronectin, to their integrin receptors. Engagement of CEACAMs, integrins and HSPGs can result in meningococcal internalization by epithelial cells. MSP: Meningococcal serine protease A; App: Adhesion and penetration protein; NadA: Neisserial adhesin; NhhA: Neisseria hia/hsf homologue A.

of approximately 90 Kb, and it is bound to a gene for a stable enterotoxin[35]. For adhesion factor K-88, three plasmids have been shown to be responsible for the three known antigenic variants: K-88ab, K-88ac and K-88ad. Mooi *et al*[36], designed experiments to determine which genes of the plasmid chain were responsible for the formation of the K-88 factor in each of the variants. For this purpose, each of the three K-88 plasmids was digested with restriction enzymes, and the fragments obtained from each one were then cloned by inclusion in the PBR-322 vector. The bacterial clones carrying each of the K-88 antigens were then identified. This procedure revealed that the expression of the K-88 factor depends of the orientation of the DNA chain responsible and on the variant in question. In the case of K-88ab, its insertion into the vector PBR-322 in a direction or another modifies the quantity of antigen expressed. The lipopolysaccharide of *N. meningitidis* is known as the major determinant of its virulence, and the use of monoclonal antibodies, together with structural studies, have highlighted the heterogeneity and complexity of meningococcal lipopolysaccharides, which can be divided into 12 immunotypes[37].

Studies by McGee *et al*[38], have underlined the importance of gonococcal fimbriae in cell colonisation and destruction in cultures of cells from the human fallopian tube. These assays show that both fimbriate and non-fimbriate gonococci bind epithelial cells, although in the former case cell destruction is produced more quickly, this process being mediated by one or more toxic factors, such as surface lipopolysaccharides. Type Ⅳ pili, which are protein structures associated with the surface, have also been associated with the adhesion of *N. meningitidis* to endothelial cells and the development of fulminant meningococcal disease[39,40]. The pili of *E. coli*, which have been studied in detail, consist of protein subunits that are thought to play an important role in the interaction with specific surface carbohydrates in eukaryotic cells, and some of them are K antigens. D-(+)-Mannose inhibits the *in vitro* adhesion of bacteria with type-1 fimbriae on the surface of eukaryotic cells containing mannose residue[41]. This is an indiscriminate mechanism of adhesion, as oligosaccharide chains containing mannose are very commonly present in cell surface oligoproteins, including phagocytic cells. Preincubation of bacteria with inhibitor sugars does not affect the adhesiveness, while the pretreatment of cells with carbohydrates effectively prevents adhesion. This indicates that the cell surface structures recognise the radicals of fucose and glucose in the bacterial lipopolysaccharides.

Some authors[42,43], have analysed phenotypic changes in bacteria associated with epigenetic changes. Aspects such as virulence, response to oxidative stress and the formation of biofilm have been observed among epigenetic modifications. Unfortunately, these processes and their relationship with pathogenic changes in *N. meningitidis* are as yet incompletely understood.

Despite the high prevalence of carriers of *N. meningitidis*, it only occasionally causes meningococcal disease in the context of endemic disease, in certain geographic areas or in isolated epidemic outbreaks. Some studies have reported that oxidative stress in the environment can modify the surface characteristics

of *N. meningitidis*. Also the antigenic structure can be modified by its importing genetic material from other bacteria in its ecological niche, and some structures of lipopolysaccharides, pili and capsule change the immune response. This paper reviews current knowledge on host-environment-bacteria mechanisms and interactions, with the aim of contributing to our understanding of the pathogenic mechanisms of *N. meningitidis*.

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ORIGINAL ARTICLE

Observational Study

Improvement in human immunodeficiency virus-1/acquired immune deficiency syndrome patients' well-being following administration of "Phyto V7"

Ruben Wernik, Jose L Priore, Walter F Goldman, Adriana del Carmen Elias, Gadi Borkow

Ruben Wernik, Facultad de Medicina, Universidad de la República, Montevideo CP 11800, Uruguay
Jose L Priore, Uruguay Servicio Médico Penitenciario, Dirección Nacional de Cárceles, Penitenciarías y Centros de Recuperación, Montevideo CP 11100, Uruguay
Walter F Goldman, Gadi Borkow, Immune Nutrition Incorporated, Gibton 76910, Israel
Adriana del Carmen Elias, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, San Miguel de Tucumán, Tucumán 4000, Argentina

Author contributions: Wernik R and Goldman WF were involved in the design of the study and interaction with the Uruguay Government and General Direction of Prisons; Priore JL was in charge of the actual implementation of the trial; Elias AC and Borkow G analyzed the data and wrote the manuscript.
Ethics approval: The protocol was reviewed and approved by the Ethical Medical Committee of the Ministry of Health of Uruguay.
Informed consent: All study participants provided informed written consent prior to study enrollment.
Conflict-of-interest: Dr. Walter F Goldman and Dr. Gadi Borkow are members of the Immune Nutrition Incorporated, the company that produces the Phyto V7 complex. All other authors do not have a conflict of interest.
Data sharing: Technical appendix, statistical analyses, and dataset are available from the corresponding author at dr.borkow@gmail.com. Consent was not obtained but the presented data are anonymized and risk of identification is nonexistent.

Correspondence to: Dr. Gadi Borkow, Chief Scientist, Immune Nutrition Incorporated, Hameyasdim 44, Gibton 76910, Israel. dr.borkow@gmail.com
Telephone: +972-546-611287
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Abstract

AIM: To corroborate the capacity of Phyto V7, a complex of phytochemicals, to improve the physical well-being of human immunodeficiency virus-1 (HIV-1) infected and acquired immune deficiency syndrome (AIDS) patients not undergoing antiretroviral treatment.

METHODS: Two hundred and thirty nine HIV-1 sero-positive male and female voluntary inmates were recruited through the Uruguay National Program of AIDS. The study participants received for 90 consecutive days every eight hours two tablets (760 mg/each) of Phyto V7, containing a mix of the following phytochemicals: flavonols (Kaempferol, Quercetin), flavones (Apigenin, Luteolin), hydroxycinnamic acids (ferrulic acid), carotenoids (Lutein, Lycopene, Beta carotene) and organosulfur compounds, all from vegetal origin. The participants did not receive any antiretroviral treatment during the study. At days 0, 30, 60 and 90 (± 2 d) the participants were evaluated for body mass index (BMI), tolerance to Phyto V7 and Index of Quality of Life based on the Karfnosky scale. ANOVA, Tukey Post-test, χ^2 test and Wilcoxon Signed Rank test were used to analyze the effect of treatment.

RESULTS: One hundred and nighty nine study participants finished the study. Already after 30 d of Phyto V7 consumption, the weight, BMI and Karnofsky score statistically significantly improved ($P < 0.001$), and continued to improve until the end of the study. The mean weight gain per participant during the 90 d was

of 1.21 kg (approximately 2% of body weight). The overall increase in the mean Karnofsky score after 90 d was 14.08%. The lower the BMI and Karnofsky score of the participants were at the beginning of the study, the more notorious was the improvement over time. For example, the mean increment of Index of Quality of Life, among the participants with an initial Karnofsky score of 5 or below (n = 33) from day 0 to day 90, was of 35.67% (0.476 ± 0.044 *vs* 0.645 ± 0.09; P < 0.001). The tolerability to Phyto V7 was very good and no adverse reactions were recorded or reported.

CONCLUSION: Administration of the Phyto V7 can be an important tool to improve the well-being of HIV-1 seropositive individuals and AIDS patients, not undergoing antiretroviral treatment.

Key words: Phytochemicals; Karnofsky score; Nutrition; Human immunodeficiency virus-1; Acquired immune deficiency syndrome

Core tip: Phyto V7 is a complex of phytochemicals and micronutrients. Phyto V7 has been found to stimulate the immune system and dramatically improve the physical well-being of terminal acquired immune deficiency syndrome (AIDS) patients. The current study demonstrates the capacity of Phyto V7 to improve the physical well-being of human immunodeficiency virus-1 (HIV-1) infected and AIDS patients not undergoing antiretroviral treatment, as demonstrated in 199 individuals. We conclude that administration of the food supplement Phyto V7 can be an important tool to improve the well-being of HIV-1 seropositive individuals and AIDS patients, not undergoing antiretroviral treatment.

Wernik R, Priore JL, Goldman WF, Elias AC, Borkow G. Improvement in human immunodeficiency virus-1/acquired immune deficiency syndrome patients' well-being following administration of "Phyto V7". *World J Clin Infect Dis* 2015; 5(2): 44-50 Available from: URL: http://www.wjgnet.com/2220-3176/full/v5/i2/44.htm DOI: http://dx.doi.org/10.5495/wjcid.v5.i2.44

INTRODUCTION

The energy needed for physical activity and for maintaining the body weight is higher in human immunodeficiency virus-1 (HIV-1) infected individuals than in non-HIV infected individuals[1,2]. Acquired immune deficiency syndrome (AIDS) patients spend approximately 20% to 30% more energy than healthy individuals in order to maintain their body weight, including when receiving highly active antiretroviral treatment (HAART)[3,4]. The World Health Organization has recommended including micronutrient supplementation as an integral part of all HIV treatment programs[5]. Micronutrient supplementation trials demonstrated a reduced mortality and improved clinical outcomes in HIV-1 infected individuals, regardless of their clinical stage and use of antiretrovirals[6-9].

Phytochemicals, chemical compounds that occur naturally in plants, in addition of serving as micronutrients, enhance nonspecific immunity[10], down regulate inflammatory diseases[11], possess radical scavenging activities[12], and inhibit disease progression[13-19]. For example, administration of phytochemicals reduced hepatotoxic, lithic, and hepatitis related adverse symptoms[19]. Some phytochemicals inhibit HIV-1 protease and integrase, and inhibit viral entry to target cells[12,20-24]. Phyto V7 is a complex of phytochemicals, which also contains micronutrients, registered as a nutritional supplement in several countries. Administration of Phyto V7 to chicks enhances their humoral immune responses against Newcastle Disease Virus following vaccination[25]. Furthermore, its administration to human papilloma virus (HPV) affected women undergoing electrosurgical excision of cervical lesions resulted in approximately two-fold higher elimination of HPV than in the control group of women. In the group of woman receiving Phyto V7 there was an increase in the local cellular immune responses, as exemplified by much higher elevated presence of NK cells and cytotoxic T-cells ($CD8^+$) in the cervical smears 90 d after the electrosurgical excisional procedure[26]. We have also found an increase in $CD4^+$ T-cells in HIV-1 infected individuals taking Phyto V7, without affecting their viral loads titers (manuscript in press). Taken together, the above findings indicate that Phyto V7 has immune-stimulatory properties. Remarkably, administration of Phyto V7 to 9 terminally ill AIDS patients resulted in a dramatic improvement in their physical status[27].

Antiretroviral treatment, which can effectively control viremia, requires high patient adherence for life. Low patient adherence results in the appearance of drug resistant viral isolates and necessitates different treatment protocols and salvage therapy options. Unfortunately, in many developing countries HIV-1 infected individuals are not treated at all. Many reasons account for that, such as inappropriate or non-existent centralized government treatment programs and elevated costs of antiretroviral treatments. One of the treatment neglected populations, in many developing countries, is prison inmates. The rates of HIV-1 infection are very high in this population[28,29]. Prison inmates are at higher risk of HIV-1 infection due to increased intravenous drug use, unprotected sexual activity, exposure to blood during fights, and tattooing.

In the current manuscript we report the very significant improvement in the well-being of 199 HIV-1 infected prison inmates, who did not receive any antiretroviral treatment while in prison, receiving only a daily administration of Phyto V7.

MATERIALS AND METHODS

The methodological design of the study was analytical

Table 1 Karnofsky score used

10	No complaints, no signs of disease
9	Capable of normal activity, few symptoms or signs of disease
8	Normal activity with some difficulty, some symptoms or signs
7	Caring for self, not capable of normal activity or work
6	Requiring some help, can take care of most personal requirements
5	Requires help often, requires frequent medical care
4	Disabled, requires special care and help
3	Severely disabled, hospital admission indicated but no risk of death
2	Very ill, urgently requiring hospital admission, requires supportive measures or treatment
1	Moribund, rapidly progressive fatal disease processes

Table 2 Frequency of increase in the weight of the study participants over time

Weight	30 d		60 d		90 d	
	n	%	*n*	%	*n*	%
Decrease	27	13.6	19	9.5	12	6
Equal	65	32.7	36	18.1	24	12.1
Increase	107	53.8	144	72.4	163	81.9

and longitudinal, conducted by mid-2010 in Uruguay through the patronage of Dr. Tabaré Vasquez, President of Uruguay, by the General Direction of Prisons and the Uruguay Association of Seropositives. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was reviewed and approved by the Ethical Medical Committee of the Ministry of Health of Uruguay. HIV-1 seropositive male and female inmates were recruited from the Libertad, La Tablada and Cabildo prisons. All study participants gave their informed consent prior to the commencement of the study.

Phyto V7 was donated by the Israel Project Life Foundation and Immune Nutrition Incorporated. Phyto V7 was registered as a food supplement (Registration Number 54221) at the Division of Health Products, Department of Food. Each Phyto V7 tablet contained 760 mg of the following phytochemicals: flavonols (Kaempferol, Quercetin), flavones (Apigenin, Luteolin), hydroxy-cinnamic acids (ferrulic acid), carotenoids (Lutein, Lycopene, Beta carotene) and organosulfur compounds, all from vegetal origin.

During the study, each participant was given every 8 h two Phyto V7 tablets. At days 0, 30, 60 and 90 (± 2 d) the participants were evaluated for body mass index (BMI), tolerance to Phyto V7 and well-being. The well-being was estimated according to the modified Karfnosky scale (Table 1). Each time the doctor in charge filled the questionnaire while examining and consulting each study participant, without seeing the previous already filled questionnaires. No data regarding the viral load or immune profile of the participants could be gathered. The participants did not receive any antiretroviral treatment during the study.

The differences between weight and BMI were analyzed with Kruskal-Wallis One Way Analysis of Variance on Ranks (ANOVA) and Tukey Post test. The proportions of levels of quality of life were analyzed with the χ^2 test. An Index of Quality of Life was defined by dividing the levels of the Karnofsky score by the maximal level (10) and applied the Wilcoxon Signed Rank Test to analyze the differences. The SigmaPlot 12 software was used to conduct the statistical analyses.

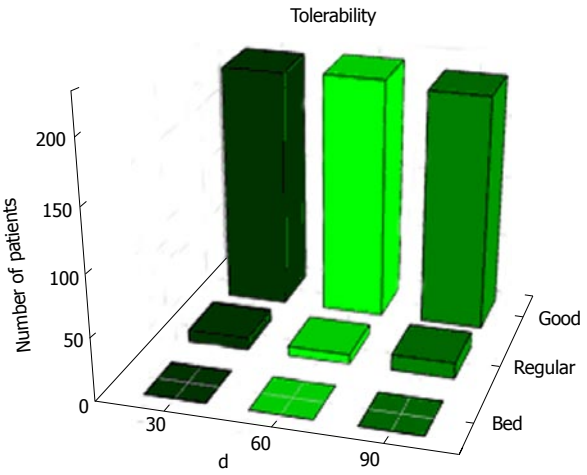

Figure 1 Assessment of Phyto V7 tolerability. The assessment of tolerability is based on the medical examination and the participant's feedback and general feeling.

RESULTS

A total of 239 HIV-1 seropositive inmates were recruited. Forty participants did not finish the study due to various reasons, such as being transferred to other facilities and being released from prison. Thus, the data presented is of 199 participants.

As reported by the study participants, after taking Phyto V7 for 30, 60 and 90 d, the tolerability to Phyto V7 was very good (Figure 1). No adverse reactions were recorded or reported.

As can be seen in Table 2, the proportion of individuals that participated in the study in whom there was an increase in their weight was 53.8%, 72.4% and 81.9% after 30, 60 and 90 d, respectively. The increase in weight was statistically significant ($P < 0.001$). After 90 ds there was a decrease in weight in only 6% of the patients. The increase in the mean weight of the study participants can be appreciated in Figure 2B. The mean weight gain per participant during the 90 d was of 1.21 kg (approximately 2% of body weight).

In accordance with the increase in the weight, also the BMI of the participants increased over time (Figure 3A). The mean of BMI increased from 23.18 on day 0 to 23.64 on day 90, a 1.98% increase. When analyzing the mean increase in the BMI of the group of participants that had a BMI of below 21 at the beginning of the study ($n = 60$), the increase in BMI is even more impressive (Figure 3B) - the mean in BMI among this group increased from 19.69 on day 0 to 20.24 on day 90, a 2.75% increase. Similarly, when

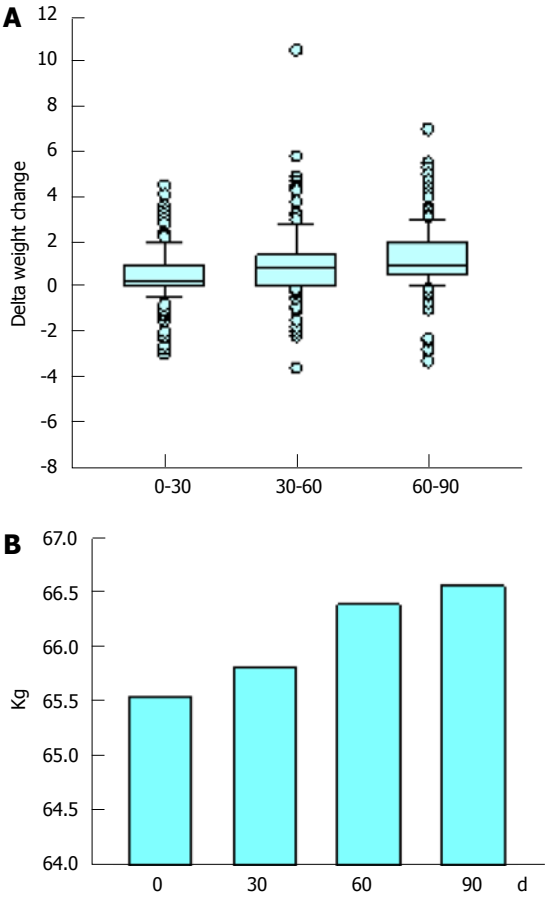

Figure 2 Participant body weight. A: Box plots describing the delta change in the weight of the study participants. The boxes represent the middle 50% of the data values. The horizontal line across the box marks the median value. The error bars show the 10th and 90th percentiles of the population. Individual data-points falling beyond these boundaries are shown as dots; B: The mean weight of the study participants.

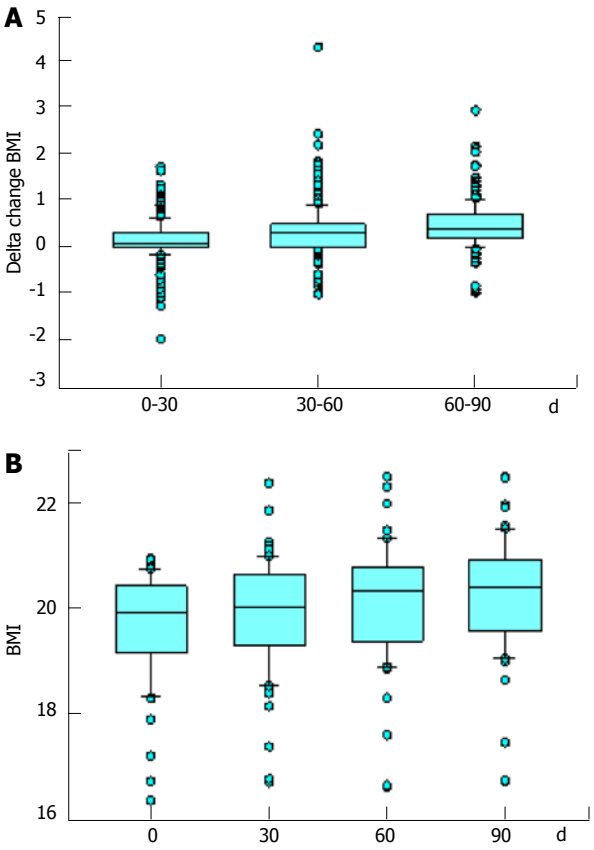

Figure 3 Body mass index of study participants. Box plots describing (A) the delta change in body mass index (BMI) of all the study participants over time and (B) the BMI of the participants who had a BMI of less than 21 at the onset of the study.

looking into the 11 participants that had a BMI of below 19 at the beginning of the study (n = 11), the mean BMI increased from 18.02 on day 0 to 18.62 on day 90, a 3.04% increase.

The overall quality of life of the participants increased over time, as determined by the Karnofsky scale determinations, and as exemplified for the Index of Quality of Life in Figure 4A. The Index of Quality of Life was already statistically significantly higher at day 30 compared to day 0 (mean of 0.657 *vs* 0.632; $P < 0.001$). The Index of Quality of Life continued to increase with Phyto V7 consumption, from a mean of 0.657 to 0.7 and 0.721 at days 30, 60 and 90, respectively ($P < 0.001$ between each data point). The overall increase in the mean Karnofsky score after 90 d was 14.08%.

When analyzing the changes in the Index of Quality of Life among the participants that at day 0 had a Karnofsky score of 5 or below (n = 33), the changes in the score from day 0 to day 90 are even more impressive, *i.e.,* 35.67%, from 0.476 ± 0.044 to 0.645 ± 0.09 ($P < 0.001$; Figure 4B). The clear increase in the proportions of the Karnofsky score over time is depicted in Figure 4C. For example, the level score 8 increased from day 0 to day 90 by approximately 5 fold, from 7.5% to 36.7% for all study participants. In contrast, the level score 5 decreased from day 0 to day 90 from 13.1% to 1.5%.

At day 90, approximately 73% of the study participant's felt that consumption of Phyto V7 was beneficial to them, while approximately 25% felt the same. This is in accordance with an increase in weight in 81.9% of the study participants. Two percent of the patients felt that their situation worsened during the 90 d study.

DISCUSSION

Since the institution of HAART, the number of individuals becoming ill with AIDS has declined significantly and the prognosis of AIDS patients has improved notably. However, low compliance, viral cross-resistance, and significant side effects caused by HAART, serve as reason to postpone HAART. In developing countries, wide implementation of HAART may be even more problematic due to high costs, infrastructure problems and high prevalence of other ailments such as anemia and co-infections[30,31]. Thus, new, non-expensive, safe, easy to take alternative or complementary remedies, that can improve the patient's well-being, are very attractive for the treatment of individuals that fail HAART or antiretroviral naïve patients that can not get

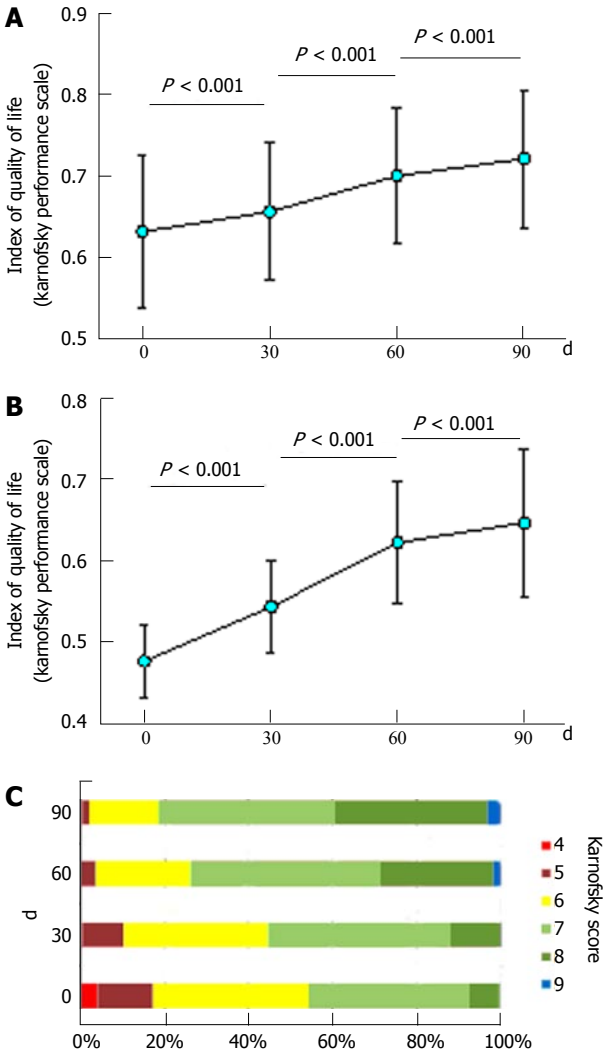

Figure 4 Quality of life of study participants based on the Karnofsky score. A: The mean and standard deviation of the Index of Quality of Life score of all study participants and of (B) participants who had a Karnofsky score of 5 or less at the onset of the study; C: The proportions of Karnofsky score at days 0, 30, 60 and 90. The *P* values of Wilcoxon Signed Rank Tests between each day are shown.

antiretroviral therapy.

Recently we published the results of a study that was conducted with 9 terminally ill AIDS patients living in a hospice[27]. All patients had very high HIV-1 viral loads and 8 out of the 9 patients were scored as C3 according to the United States Centers for Disease Control status index. Seven out of the 9 patients were antiretroviral naïve patients. During the study they did not receive antiretroviral treatment but only received the food supplement Phyto V7. While most of the patients at the commencement of the study could not eat, stand, dress or shower by themselves, after 3 mo of Phyto V7 supplementation all patients could eat, sit down, shower, stand up and dress without help. The well-being of the patients improved dramatically, both physically and mentally. The success of this trial was the incentive to conduct the current study.

As with the terminally ill AIDS patients, the administration of Phyto V7 to HIV-1 infected, asymptomatic and symptomatic individuals in the current study, resulted in a very significant improvement in the individuals' well-being. The weight, BMI and Karnofsky score of the study participants increased notably, especially in those who had a low BMI and low Karnofsky score at the onset of the study. Increase in appetite, weight, and individuals mood, has a positive outcome in the individual well-being. Notably 83% of the participants adhered until the end of the trial and took Phyto V7, indicating the high likelihood that they will continue using Phyto V7 also finalizing the study. Part of the positive effect of Phyto V7 can also be explained as phytochemicals having radical scavenging activities[12], stimulating nonspecific immunity[10], and down regulating inflammatory responses[11]. Indeed, Phyto V7 has been shown to enhance humoral and cellular immune responses[25,26]. It is not clear from this study if crucial parameters relevant to the progression to AIDS were affected, such as the $CD4^+$ T-cell counts and viremia. However, in a another study (manuscript in press) the administration of Phyto V7 resulted in the upregulation of $CD4^+$ T-cell counts without affecting viral loads, indicating that Phyto V7 has an immuno-stimulating effect and no direct antiviral effect.

Administration of a food supplement, such as the Phyto V7, is extremely inexpensive as compared to HAART. Phyto V7 is from a natural source and as opposed to antiretrovirals, does not affect directly HIV-1. Thus its uptake with low adherence would not result in appearance of drug resistant viruses. Obviously, in order to increase its efficacy, high compliance is desired. Administration of Phyto V7 may potentially postpone the need to treat HIV-1 infected individuals with HAART, postponing the potential complications associated with this treatment. It may well be that Phyto V7 can be given in conjunction with HAART resulting in better prognosis. These assumptions need to be examined in placebo controlled studies.

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COMMENTS

Background

The immune system of human immunodeficiency virus-1 (HIV-1) infected individuals decays with the progression of time until they develop immu-

nodeficiency. HIV-1 infected individuals also have increased energy needs than non-HIV infected individuals and many suffer from significant weight loss and wasting. Micronutrient supplementation is thus recommended as an integral part of all HIV treatment programs.

Research frontiers

Micronutrient supplementation improves the physical condition of HIV-1 infected individuals and acquired immune deficiency syndrome (AIDS) patients regardless of their clinical status and antiretroviral treatment, as was demonstrated in several studies. The administration of micronutrients that also enhance the immune system may be significantly advantageous to the HIV-1 infected individuals.

Innovations and breakthroughs

The food supplement Phyto V7 is a complex of phytochemicals and micronutrients. Phyto V7 has been found to stimulate cellular and antibody immune responses against viruses both in humans and in chicks. Importantly, administration of Phyto V7 to 9 terminal AIDS patients resulted in dramatic improvement in their physical well-being. The current study corroborated the significant positive effect on Phyto V7 on the physical well-being of HIV-1 infected individuals. This was demonstrated by the significant increase in the body weight and physical well-being a very large group of HIV-1 infected individuals not undergoing antiviral treatment that only received a daily dose of Phyto V7 for a period of 90 consecutive days.

Applications

Administration of the Phyto V7 can be an important tool to improve the well-being of HIV-1 seropositive individuals and AIDS patients, not undergoing antiretroviral treatment. It may well be that administration of Phyto V7 together with antiviral treatment is highly advantageous. Further studies should test this hypothesis.

Terminology

Phytochemicals are chemical compounds that occur naturally in plants. These chemicals, in addition of serving as micronutrients, have been found to enhance nonspecific immunity, down regulate inflammatory diseases, and inhibit disease progression. The Karfnosky score is a well-accepted scale used to assess the quality of life of patients. It was used by the examining physicians to address the well-being of the study participants during the study.

Peer-review

This is an interesting work with promising results in which Phyto V7, a phytocomponent mix, quickly and effectively improves the weight and makes most of the HIV patients treated to feel better. For these kinds of patients it is good to be able to help them and this treatment might prepare them for a future more aggressive antiviral therapy.

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